TO STUDY THE PREVALENCE OF HUMAN PAPILLOMAVIRUS IN THE ORAL CAVITY OF PATIENTS PRESENTING WITH CARCINOMA OF ORAL CAVITY

AT CHRISTIAN MEDICAL COLLEGE AND HOSPITAL, VELLORE.

A dissertation submitted in part fulfillment of MS Branch IV, General Surgery examination of the Tamil Nadu Dr. MGR Medical University, to be held in April 2016.

Department of General Surgery

ChristianMedicalCollege, Vellore

CERTIFICATE

This is to certify that the dissertation entitled '**The prevalence of human papillomavirus in the oral cavity of the patients presenting with oral cancers to the Christian Medical College and Hospital'** is the bonafide original work of Dr. Jennifer Prince, submitted in fulfillment of the rules and regulations for the MS Branch IV, General Surgery examination of the Tamil Nadu Dr. MGR Medical University, to be held in April 2016.

Guide:

Dr John C Muthusami
Professor and Head Unit I,
Dept. of General Surgery,
Christian Medical College,
Vellore – 632 004.

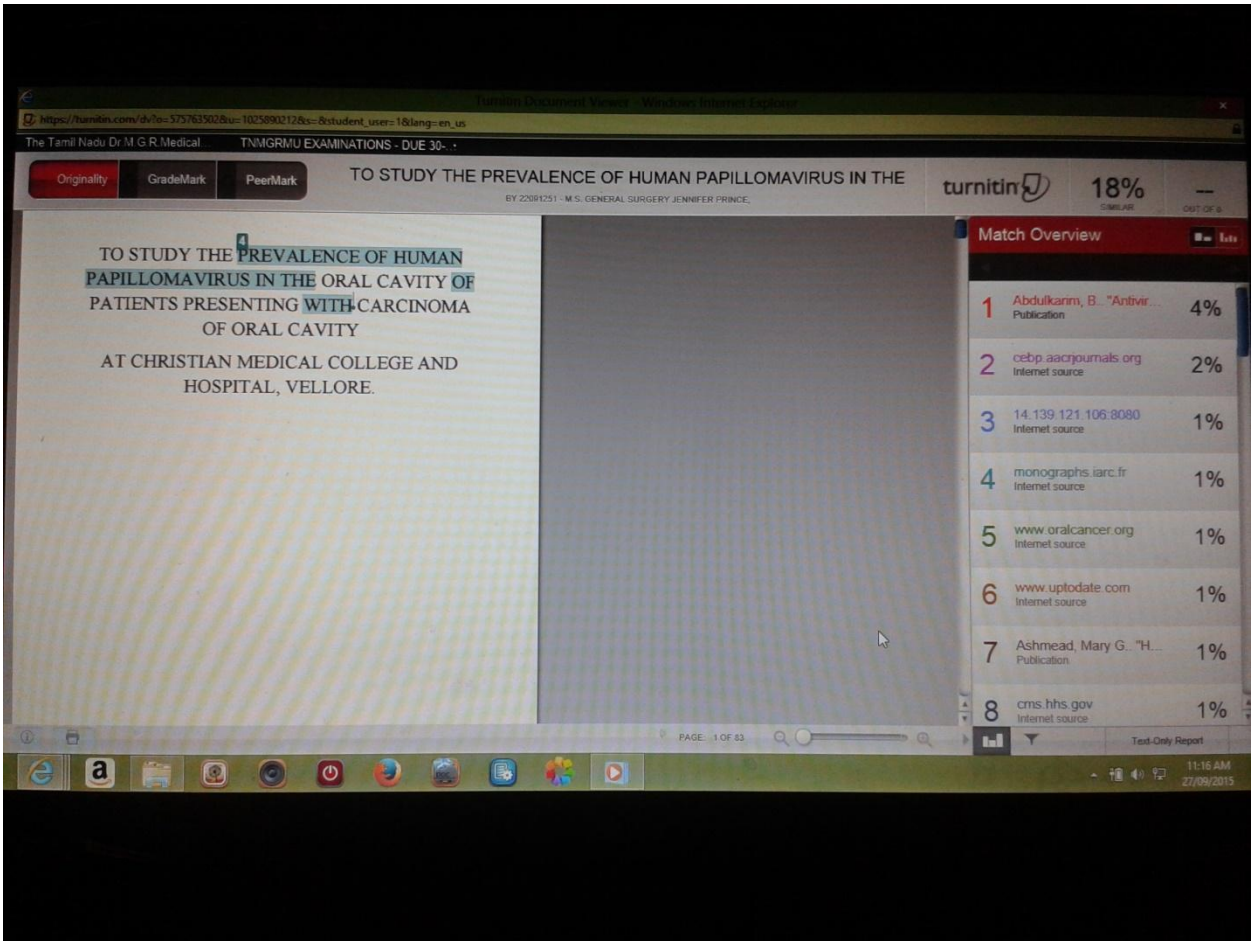
Department of General Surgery

ChristianMedicalCollege, Vellore

CERTIFICATE

This is to certify that the dissertation entitled '**The prevalence of human papillomavirus in the oral cavity of the patients presenting with oral cancers to the Christian Medical college and Hospital'**is the bonafide original work of Dr. Jennifer Prince, submitted in fulfillment of the rules and regulations for the MS Branch IV, General Surgery examination of the Tamil Nadu Dr. MGR Medical University, to be held in April 2016.

Dr. John C. Muthusami,
Head of the Department,
Dept. of General Surgery,
Christian Medical College
Vellore – 632 004


Turnitin Document Viewer - Windows Internet Explorer
https://turnitin.com/dv?o=575763502&u=1025890212&s=&student_user=1&lang=en_us
The Tamil Nadu Dr.M.G.R.Medical...
TNMGRMU EXAMINATIONS - DUE 30-...
Originality
GradeMark
PeerMark
TO STUDY THE PREVALENCE OF HUMAN PAPILLOMAVIRUS IN THE
BY 22081251 - M.S. GENERAL SURGERY JENNIFER PRINCE
turnitin
18%
SIMILAR
TO STUDY THE PREVALENCE OF HUMAN PAPILLOMAVIRUS IN THE ORAL CAVITY OF PATIENTS PRESENTING WITH CARCINOMA OF ORAL CAVITY
AT CHRISTIAN MEDICAL COLLEGE AND HOSPITAL, VELLORE.
Match Overview
1 Abdulkarim, B... "Antivir... Publication 4%
2 cebp.aacrjournals.org Internet source 2%
3 14.139.121.106:8080 Internet source 1%
4 monographs.iarc.fr Internet source 1%
5 www.oralcancer.org Internet source 1%
6 www.uptodate.com Internet source 1%
7 Ashmead, Mary G... "H... Publication 1%
8 cms.hhs.gov Internet source 1%
PAGE: 1 OF 83
Text-Only Report
11:16 AM
27/09/2015

CHRISTIAN MEDICAL COLLEGE
VELLORE - 632 002, INDIA.
INSTITUTIONAL REVIEW BOARD (IRB)

February 22, 2010

Dr. Jennifer Prince
PG Registrar
Department of General Surgery
Christian Medical College
Vellore 632 004

Sub: FLUID Research grant project NEW PROPOSAL:
To Assess the Prevalence of Human Papilloma virus in the Oral cavity of patients attending the general surgery Out Patient Department and its association with those with Oral Squamous Cell Carcinoma
Dr. Jennifer Prince, PG Registrar, General Surgery Unit I, Dr. John. C. Muthusami, General Surgery .

Ref: IRB Min. No.7096 dated 17.02.2010

Dear Dr. Prince,

The Institutional Review Board (Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project entitled "To Assess the Prevalence of Human Papilloma virus in the Oral cavity of patients attending the general surgery Out Patient Department and its association with those with Oral Squamous Cell Carcinoma" on February 17, 2010.

The Committees reviewed the following documents:

1. Format for application to IRB submission
2. Informed Consent Form (Tamil and Hindi)
3. Patient Information Sheet (English)
4. Questionnaire (English, Tamil and Hindi)
5. Cvs of Drs. John C Muthusami.
6. A CD containing document 1 – 5.

The following Ethics Committee members were present at the meeting held on February 17, 2010 at 10:00 am in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore 632002.

Name	Qualification	Designation	Other Affiliation
Dr. George Thomas	MBBS, D.Ortho	Chairperson (IRB) & Orthopaedic Surgeon, St. Isabel Hospital, Chennai &	Non-CMC Staff.

CHRISTIAN MEDICAL COLLEGE
VELLORE - 632 002, INDIA.
INSTITUTIONAL REVIEW BOARD (IRB)

		Editor, Indian Journal of Medical Ethics	
Dr. Shuba Kumar	MA, MSc, Ph.D.	Dy. Chairperson (IRB) & Social Scientist, SAMRATH, Chennai.	Non-CMC Staff.
Dr. L. Jeyaseelan	MSc, PhD, FRSS	Professor & Head, Dept. of Biostatistics & Secretary IRB (EC), CMC	
Dr. George Mathew	MBBS, MS, MD	Principal, C.M.C.	
Dr. Thambu David (on behalf of Dr. Lionel Gnanaraj)	MBBS, MS, M.Ch. (Urol)	Medical Superintendent, CMC.	
Dr. Prathap Tharyan	MD, MRCPsych.	Associate Director, Professor of Psychiatry, CMC	
Mrs. Shirley David (on behalf of Mrs. Bharathy Jacob)	M.Sc. (Nursing), RN, RM	Dean, College of Nursing, CMC.	
Rev. Dr. T. Arul Dhas	M.Sc., BD, Ph.D.	Chaplain, CMC	
Dr. Jayaprakash Muliyil	BSC, MBBS, MD, MP DrPH(Epid), DMHC	Academic Officer, CMC	
Dr. P. Zachariah	MBBS, MD	Retired Professor	Non-CMC Staff
Mr. Harikrishnan	BL.	Lawyer	Non-CMC Staff.
Mr. Samuel Abraham	MA, PGDBA, PGDPM, M.Phil, BL.	Legal Advisor, CMC.	
Dr. Sujith Chandy	MBBS, MD	Professor, Pharmacology Dept. CMC.	
Dr. Denny Fleming	MBBS, MD	Professor, Pharmacology Dept. CMC.	
Mrs. S. Pattabiraman	BSc, DSSA	Social Worker, Vellore	Non-CMC-Staff
Dr. Suresh Devasahayam	BE, MS, PhD	Professor of Bioengineering, CMC	
Dr. Gagandeep Kang	MD, PhD, FRCPath.	Dy. Chairperson (IRB), Professor of Microbiology & Addl. Vice Principal (Research), CMC.	

We approve the project to be conducted in its presented form.

The Institutional Ethics Committee / Independent Ethics Committee expects to be informed about the progress of the project, any SAE occurring in the course of the project, any changes in the protocol and patient information/informed consent and asks to be provided a copy of the final report.

A sum of Rs.60,000/- (Rupees Sixty thousand only) is sanctioned for 2 years out of which a maximum of Rs. 1,500/- can be spent for stationery, printing, Xeroxing and computer charges (if computers used are within the institution).

Yours sincerely,

L. Jeya. Ln.

Dr. L. Jeyaseelan, PhD
Secretary, IRB

Secretary

Acknowledgement:

I would like to thank the Lord, Almighty, without whose help I will not be where I am.

I would like to thank Dr. J. C. Muthusami, our HOD, who has been a source of help and tremendous inspiration to me and other post graduate students. He has been a support and a constant encouragement, in spite of our shortcomings.

I would also thank Dr. PranayGaikwad, without whose help this dissertation would never have been done.

I would also thank the other members of the unit who have helped in their own way with their words of advice and various suggestions.

Special thanks to Manju who helped me with the presentation of this dissertation.

I would like to thank my parents and my brother who always encouraged and kept me in their prayers. I would also like to thank my husband, Samuel Ajay for all his constant love, patience and support.

I would like to thank Karen and Jebu, whose home and hearts were always open, and to Liza and Meekha, who brought me so much joy.

Thanks, also to Rosh and her parents, who were there for me when I needed them most. Thanks to Jo and Gigi, Aanya and Aadya.

A heart-felt thanks to those innumerable friends who cared, who shared my dreams and fears, who supported me, even when I was unbearable. Thank you, guys.

Table of Contents

PATIENT PROFILE OF ORAL SQUAMOUS CELL CANCER IN GENERAL SURGERY OUT PATIENT DEPARTMENT IN CHRISTIAN MEDICAL COLLEGE, VELLORE.

AIM AND OBJECTIVES:

1. To look for the presence of Human Papillomavirus in the oral cavity of patients with oral squamous cell carcinoma presenting to the General Surgery Out Patient Department of Christian Medical college, Vellore and to compare it with those who do not have carcinoma of the oral cavity.

2. To study the demographic profile of the patients presenting with oral squamous cell carcinoma to the General Surgery Out Patient Department.

3. To look for an association between HPV and oral squamous cell cancer by comparing the risk factors in those with and without oral cancers.

4. To assess the the presence of Human Papillomavirus to the site of oral squamous cell carcinoma - tongue, buccal mucosa, floor of the mouth, or gingivo – buccal sulcus.

5. To compare the degree of differentiation of the tumour and the presence of Human papillomavirus.

6. To evaluate the role of other selected risk factors associated with HPV in causing oral squamous cell carcinomas.

PRESENT KNOWLEDGE

AND

REVIEW OF LITERATURE

INTRODUCTION:

Squamous cell carcinoma of the oral cavity is the sixth most common cancer in the world[1]. In the world, the commonest is found to be lung cancers in men and breast cancers in women[2].Overall, head and neck cancer accounts for more than 550,000 cases annually worldwide[3]. In India, the incidence is gradually increasing from about 30% to almost 50% in the past decade. This can be due to the increase in the chewing and smoking of tobacco among the people of India[4]. Oral cancers have been the cause of morbidity and loss of valuable patient and hospital resources.

CAUSATIVE FACTORS:

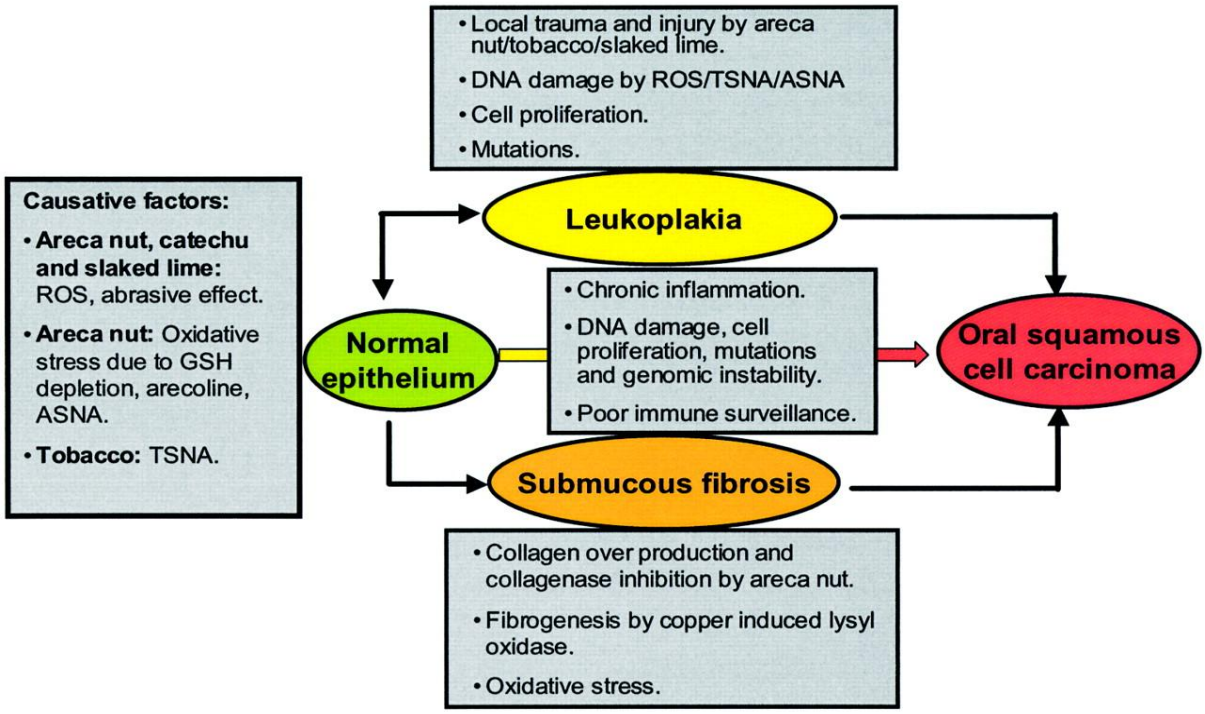
The common causes of oral cancers have been studied in great detail and the imperative nature of tobacco as a cause in the form of chewing tobacco and the smoking of cigarettes and beedis have been proven without any doubt. The risk factors most frequently associated with head and neck cancer include smoking, alcohol consumption, HPV infection (especially for oropharyngeal cancers). Many studies done in India and elsewhere have shown an increased incidence of oral cancers among those who smoke or chew tobacco. There is an increased risk of head and neck cancer, ranging from a 5- to 25-fold, in heavy cigarette smokers compared to non-smokers. There appears to be a dose-response relationship[5,6]. The main pathophysiology behind the causation has been identified as the carcinogens in the tobacco that is chewed or smoked. The relative risk (RR) in current tobacco users was 6.5. The RR increased with the duration of smoking and gradually declined after smoking cessation with no excess risk at 20 years[7].

The age of starting smoking (less than 18 years of age) and the duration of smoking (more than 35 years) were the common risk factors[8]. Cessation of smoking was associated with significant decrease in the relative risk.[9] Cigar smoking and usage of pipes are associated with an increased incidence of head and neck cancer (relative risk 2.0 to 2.6)[10,11] and smokeless tobacco (both chewing tobacco and snuff) with an increased risk of cancer of the oral cavity and pharynx.[12,13,14,15.]

Alcohol consumption independently increases the risk of cancer in the upper aerodigestive tract.But, it is often difficult to separate the effects of smoking and alcohol[16,17.] The relative risk of developing head and neck cancer due to alcohol appears to be dose dependent[17,18.] As an example, one study reported a five to six fold increased risk for head and neck cancer with alcohol intake greater than 50g/day versus less than 10g/day (one drink contains approximately 12g of alcohol.)[18]. Alcohol intake and tobacco smoking appear to have an interactive and multiplicative effect on the risk of developing head and neck cancer.[16,17,18.]

Another common cause is due to chronic irritation to the oral mucosa.[19.] This has been found true in cases of carcinoma of the lateral border of the tongue where there may be chronic irritation due to chipped teeth or ill- fitting dentures.[20] The odds ratio has been found to be 3.4 as compared with those without any dental problems[19]. The continuous irritation to the mucosa leads to dysplastic changes which later turn malignant.

There are many factors in play in the causation of oral cancers. This mainly involves the dysplastic changes caused in the normal epithelium. There are many premalignant conditions that occur which include leukoplakia, erythroplakia and sub mucous fibrosis which in turn lead to malignancy. The following picture depicts the changes that occur in the normal epithelium leading to cancer formation.



HUMAN PAPILLOMA VIRUS AND OROPHARYNGEAL CANCERS:

Of recent interest and often studied entity with regard to the causation of oral squamous cell cancers is the presence of Human Papillomavirus.[5,6,21,22,23.] Multiple types of viral infections have an established relationship with increased risk of head and neck cancer, includingEbstein-Barr virus (EBV) and Human Papillomavirus (HPV).

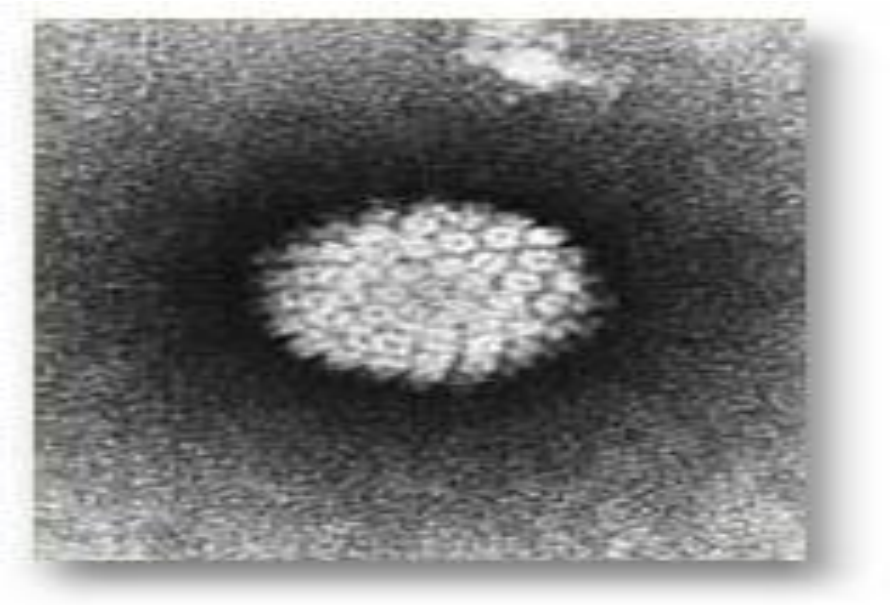
Epidemiological and molecular evidence has established a causal role for HPV, primarily those arising in the base of tongue and tonsils. These HPV associated head and neck cancers are seen in younger men, who are typically non-users of tobacco and alcohol. HPV is found in the oral mucosa and is considered causative in some of the cases, especially in tumours of the Waldeyer's ring.[24]

Cohort studies from the 1990s suggested that approximately 50 percent of oropharyngeal cancers were attributable to HPV, while more recent studies suggest that HPV may account for as much as 70 to 80 percentage of these malignancies.[25,26]

HUMAN PAPILLOMAVIRUS:

Human Papillomavirus is a double-stranded DNA virus that infects the epithelial cells of skin and mucosa. It is made up of approximately 7900 base pairs. DNA sequencing techniques have facilitated HPV typing and characterization with each type formally defined as distinct having less than 90 percent DNA base pair homology with any other HPV type.[27]

The moist epithelial surfaces (squamous cells) include all areas covered by skin and /or mucosa such as the mouth, throat, tonsils, vagina, penis and anus.



The above is a picture depicting the Human Papillomavirus.[28]

Transmission of the virus occurs when these areas come into contact with a virus, allowing it to transfer between epithelial cells. While it is now established that sexual contacts both conventional and oral are means of transferring the HPV virus, it is still poorly understood what other transfer pathways may exist.[29]

CARCINOGENESIS AND HUMAN PAPILLOMAVIRUS

There is a need for further knowledge of Human Papillomavirus in the causation of oral cancers, as DNA viruses have been found to be causative agents in multiple cancers, examples like Ebstein Barr Virus which causes Burkitt's lymphoma in people of Africa, nasopharyngeal carcinoma, some T-cell and B-cell lymphomas, and 50% of Hodgkin's lymphomas.With increasing incidence of carcinoma cervix and its proven association with Human Papillomavirus[30], there is now the possibility of prevention of this cancer using vaccines. Since the oral squamous epithelium is similar in structure to the cervical epithelium, it has been postulated that there may be a common causative organism, namely HPV[30].

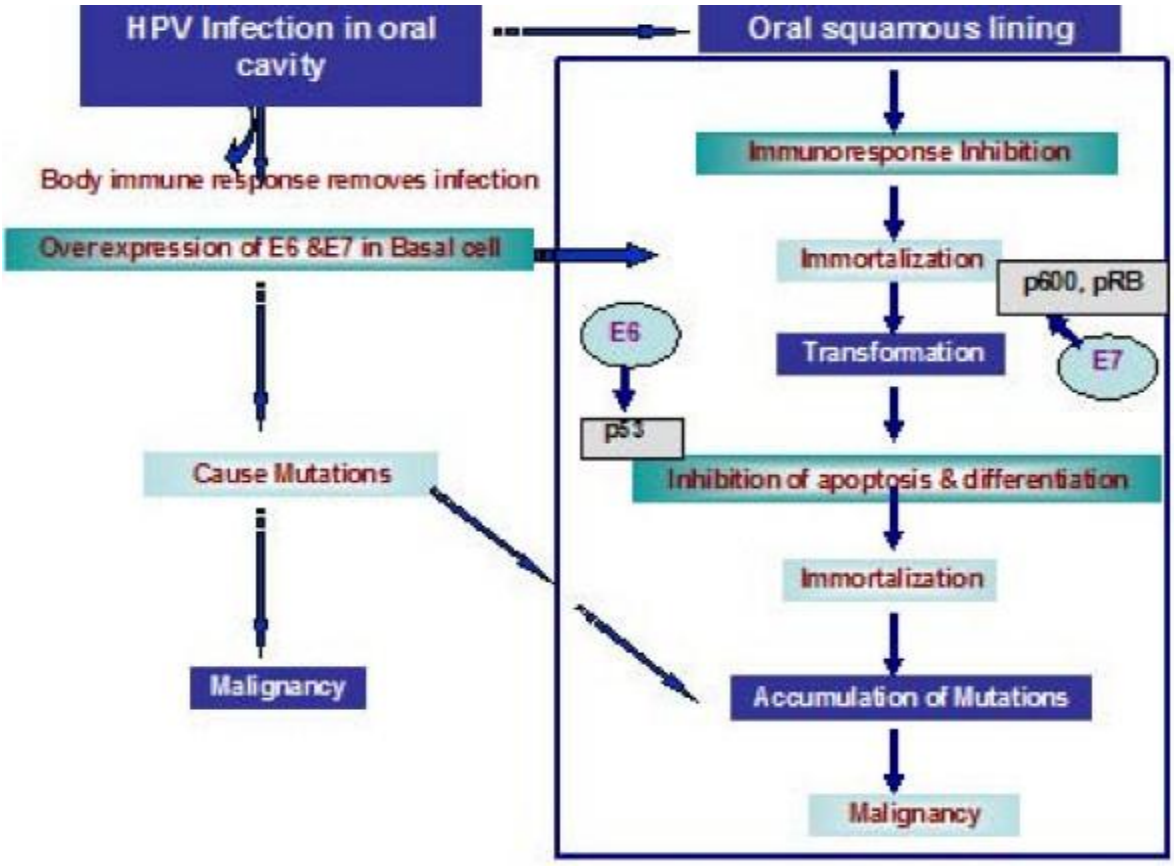


Fig.1 Mechanisms of HPV-associated oral carcinogenesis

The carcinogenic properties of HPV have been well studied and have been proved in the carcinoma cervix[30]. The pathogenesis of this cancer includes HPV oncoproteins and their interactions with host cellular oncoproteins. The oncoproteins E6 and E7 are consistently expressed in HPV-carrying anogenital cancers.These (E6 and E7) decrease the death of human keratinocytes and mammary epithelial cells, lowering the growth-factor requirement of these cells, thereby making them divide without stopping.

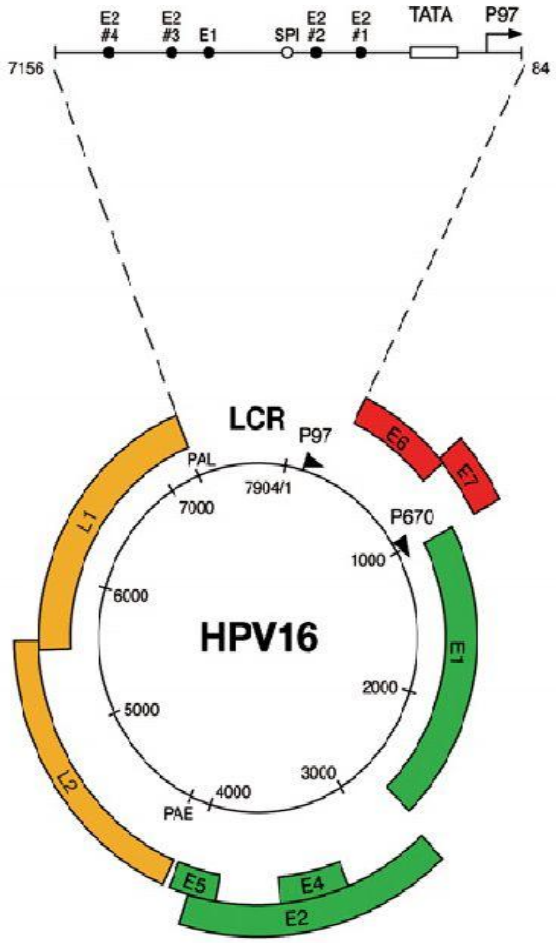
MOLECULAR CARCINOGENESIS[31]:

Carcinogenic progression of lesions infected with HPV16 or HPV18 has been associated with integration of the viral genome into the host cell's chromosomes. The integration occurs in a way that results in the loss of viral E1 and E2 gene expression (disruption of open reading frames), whereas the E6 and E7 (the oncoproteins) open reading frames frequently remain intact and are actively transcribed. In contrast to the monocistronic mRNA that encodes E6 and E7 from low-risk HPV (HPV6 and HPV11), E6 and E7 from high-risk HPVsare produced as a bicistronic message.

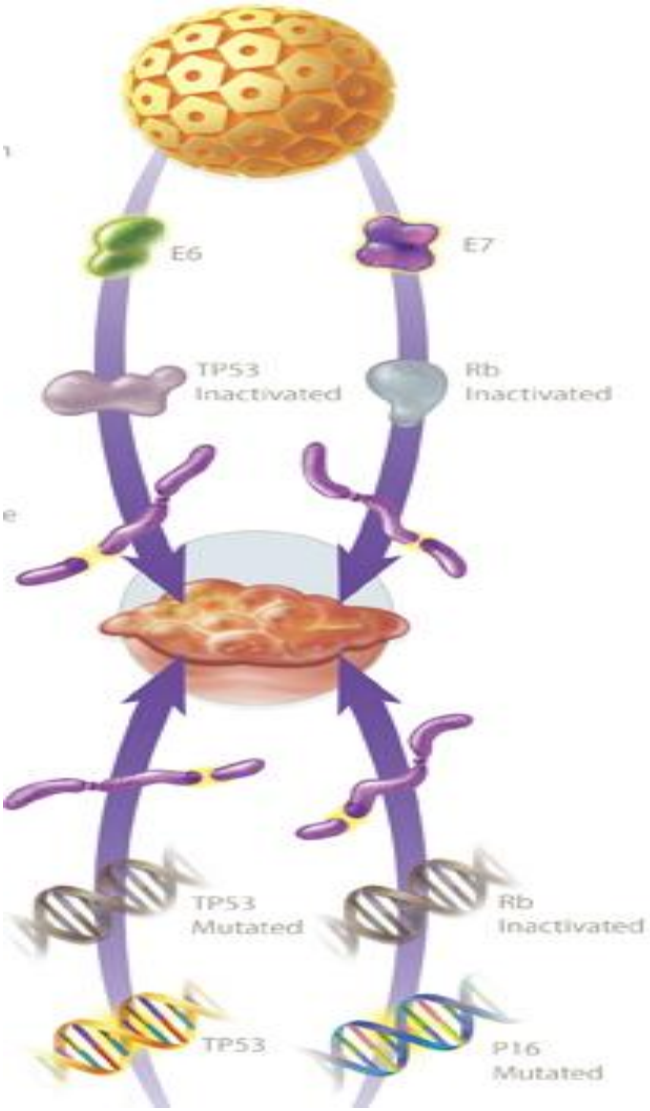
The transforming properties of HPVs are due to the interaction of viral oncoproteins with cellular proteins that control cell proliferation and apoptosis. The tumour suppressor proteins P53 and PRb are key regulators of cell cycle progression. P53 acts by mediating the G1/S checkpoint through transactivation of P21, a cyclin-dependent kinase inhibitor. PRbacts by sequestering E2F, a transcription factor that brings about the transcription of genes essential for DNA synthesis. E6 protein binds to P53 tumour-suppressor protein through

its interaction with E6-associated protein ligase, leading to the ubiquitin-dependent degradation of P53. This prevents the accumulation of P53 in cells. Thus, HPV is able to overcome its growth-arrest and apoptosis-inducing functions. Also, in *P53*-null mice, HPV E6 still prevents the induction of apoptosis[31].

The proapoptotic protein BAK, from the BCL2 family, is expressed highly in the upper layers of epithelium. This is also the site of HPV replication. E6 can inhibit BAK-induced apoptosis. This explains the chromosomal instability of cells infected with high-risk HPV types, leading to carcinogenesis. The HPV E7 protein can induce growth in various established rodent fibroblast line and acts along with E6 to delay, or even prevent cell death of primary human keratinocytes. This activates mutational consequences for the cells. These mutations are partly explained by the ability of E7 to interact with and to induce destabilisation of the 'pocket' proteins PRb, P107, and P130. These proteins negatively regulate the activity of several transcription factors, including members of the E2F family. This is done by direct association. This interaction is a critical factor in uncontrolled growth of cells infected with high-risk HPVs. In addition, E7 oncoproteins inactivate the inhibitors of cyclin-dependent kinase P21 and P27[31].

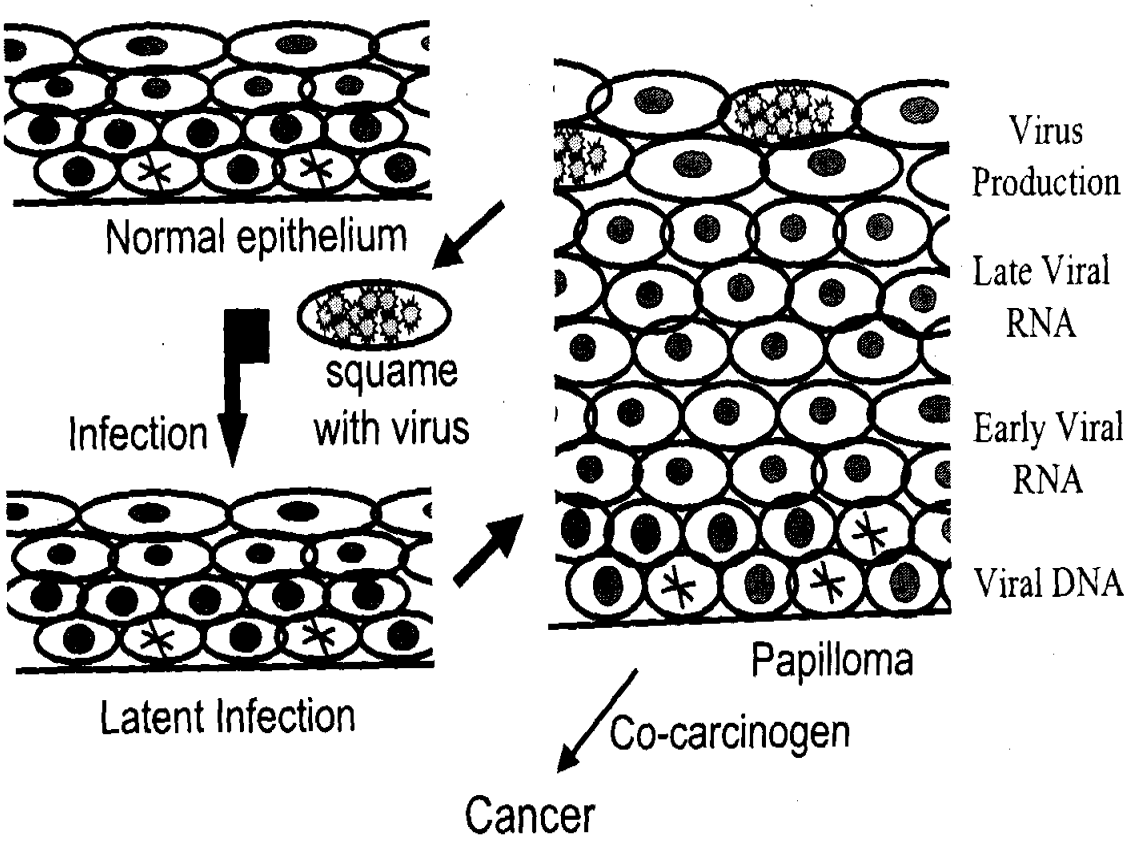


This picture depicts the various oncoprotein ligases that disrupt the normal cell pathways and lead to dysplasia.

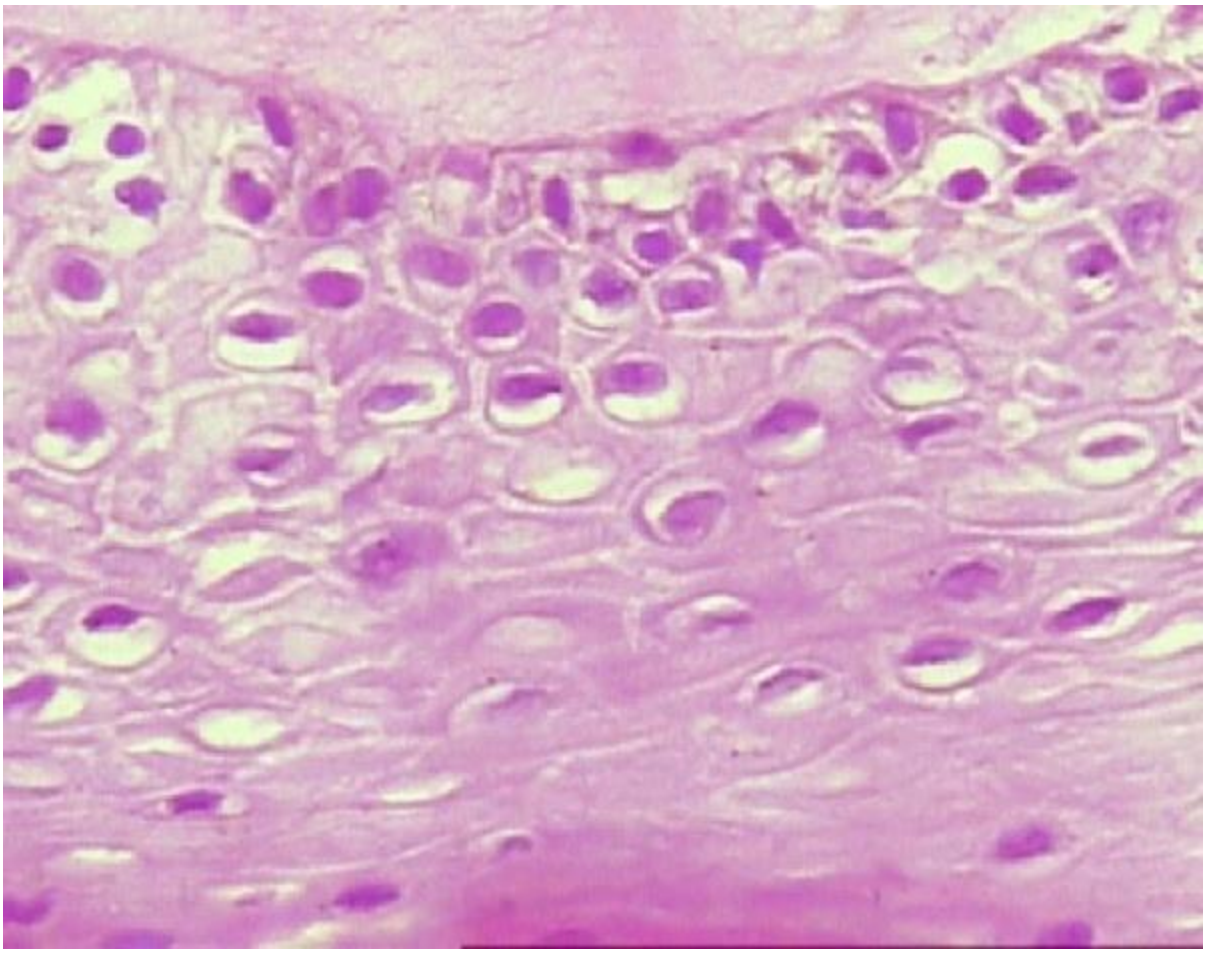


Therefore, E7 has the ability to deregulate the cell cycle. Along with this, E7 promotes apoptosis(cell death) in cells expressing wild-type P53. Expression of E7 in normal fibroblasts or in human keratinocytes induces typical markers of apoptosis. Thus, the Human Papillomavirus affects the cell cycle causing increased proliferation of cells and inhibits apoptosis, thereby leading to dysplasia which leads on to malignancy.

Cytological and histopathologically presence of Koilocytes can indicate the presence of Human Papillomavirus.



PICTURE DEPICTING THE PROGRESSION FROM INFECTION WITH HPV TO CARCINOGENESIS



PICTURE DEPICTING KOILOCYTES IN THE MUCOSAL EPITHELIUM WHICH IS CHARACTERISTIC OF HPV INFECTION.

CLINICOPATHOLOGICAL FEATURES:

It is seen that the oral cancers which are positive for Human Papillomavirus differ from the regular oral cancers in a few features. Patients with HPV positive oropharyngeal cancer are approximately 10 years younger when compared to HPV negative patients.[32,33.]

HPV associated tumors predominantly arise in the base of the tongue or the tonsillar region, although a small percentage of tumors at other sites are also HPV positive[1,33,34.] Multiple studies have shown that HPV associated oropharyngeal cancer is more likely to present with a relatively early stage (T1/T2) primary tumor, but relatively advanced disease in the neck (N2/N3)[33,34.]

HPV testing falls into three main categories[35]:

- **HPV DNA testing** – HPV DNA testing was the first approach developed for routine clinical testing. Many studies have shown that the addition of HPV DNA testing to cervical cytology improved the sensitivity for detection of cervical cancer precursors, such as cervical intraepithelial neoplasia (CIN) 2 and 3.
- **HPV RNA testing** – HPV RNA testing, where expression of E6 and/or E7 RNA are looked for, may be performed with the expectation that active HPV oncogene expression would provide better sensitivity and specificity than HPV DNA testing. Studies done this far indicate that some of these tests do result in similar sensitivity to HPV DNA testing with slightly higher specificity. None of these tests are currently FDA-approved, although this is likely to change in the near future.

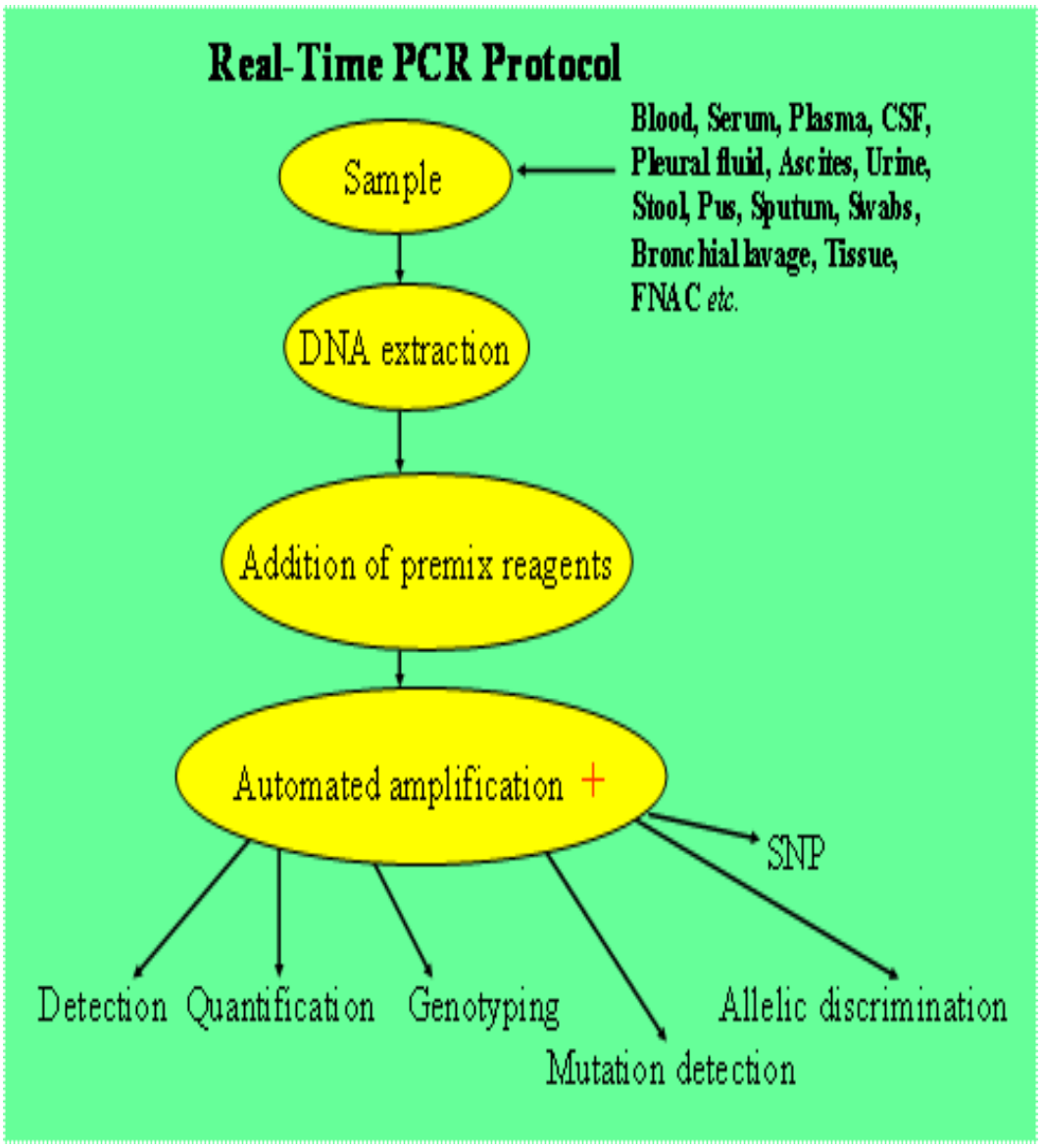
- **Detection of cellular markers** – Cellular marker detection uses a different approach to diagnosing HPV-associated disease. The HPV E7 protein disrupts cell cycling leading to an increase in cellular p16 protein expression. High-grade CIN lesions contain high levels of p16, and pathologists often immunostain cervical biopsies to help distinguish between high-grade CIN and immature squamous metaplasia. This is not associated with HPV and is not precancerous.

The method we used was the Real time Polymerase Chain Reaction[36]. The procedure follows the general principle of polymerase chain reaction; its key feature is that the amplified DNA is detected as the reaction progresses in real time. This is a new approach compared to standard PCR, where the product of the reaction is detected at its end. Two common methods for detection of products in real-time PCR are:

(1) non-specific fluorescent dyes that intercalate with any double-stranded DNA, and

(2) sequence-specific DNA probes consisting of oligonucleotides that are labeled with a fluorescent reporter which permits detection only after hybridization of the probe with its complementary DNA target.

Frequently, real-time PCR is combined with reverse transcription to quantifymessenger RNA and Non-coding RNA in cells or tissues.



This is a rough algorithm that explains the basic steps in real time PCR amplification.

The steps involved in a RT-PCR are described below:

1. The first step is to raise the reaction temperature and melt the dsDNA.
2. The temperature is then decreased so that specific primers bind to the sequences at each end of the target DNA.
3. The intervening DNA is then synthesized by DNA polymerase reaction in opposite direction. As a result of this, we have two double strand copies of the target DNA when we started with only one.
4. To detect the generation of new amplicons in RT-PCR, the PCR reaction requires an additional ingredient, a single stranded DNA probe which is designed to hybridise to the part of the DNA sequence synthesized between the two primers.
5. This probe is more defined in a special way where one of its nucleotides is labeled covalently with a fluorescent molecule and another nucleotide is labeled with a fluorescent quenching molecule. The quencher rapidly absorbs any light emitted by the fluorescent molecule as long as it remains in close proximity.
6. As the primers bind to the separate strands of DNA, the probe also finds its complimentary sites between them. The enzyme that synthesize new DNA from the end of the primers also has a second activity; an exonuclease activity, so when it encounters dsDNA in its path, it will disassemble the strand that is in its way and replace all the nucleotides.

7. As the polymerase catch through the probe, the nucleotide bearing the fluorescent marker and the one bearing the quencher are separated from one another. In the absence of a nearby quencher, the fluorescent molecule can now emit detectable

light when it is stimulated.

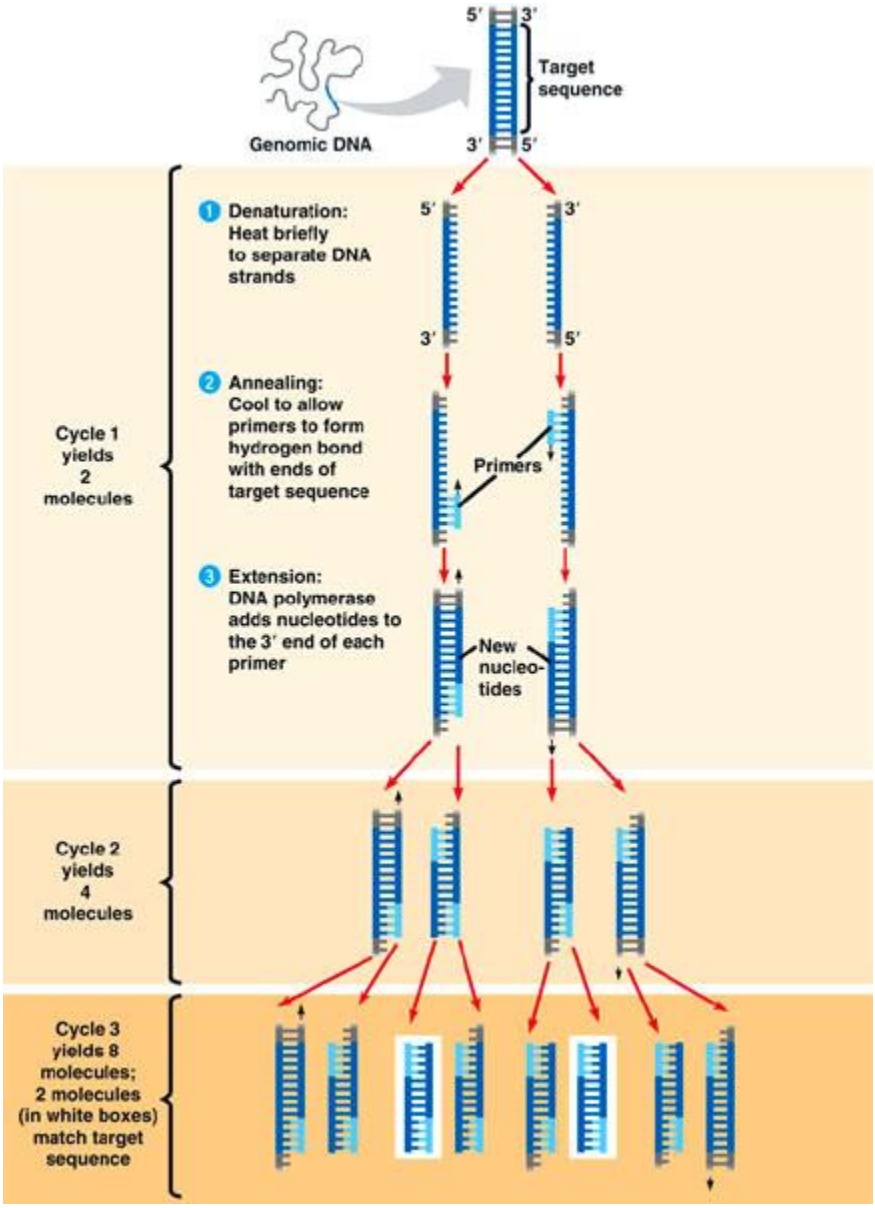
8. The next step involves the detection and measurement of the light signal. Each time another amplicon is produced, another fluorescent marker is released from its neighbouring quencher. Therefore, just as the number of amplicons doubles in each PCR cycle, the amount of emitted fluorescent energy also doubles.

9. This light generation can be monitored during the PCR reaction in a thermostat lab that is equipped with a fluorometer. When we begin with a clinical sample that had only one copy of the target DNA, it can take a few more cycles before the amplions are detected by a fluorometer in a specialized thermocycle. So, the amount of specific DNA in the clinical sample is determined by the reference to the rounds of PCR in which the amount of fluorescence first crosses the threshold of detection.

10. In cases of viruses with no DNA, the viral RNA from the RNA virus can be quantified after it is copied first and is converted to dsDNA

11. In this case, the RNA is released from the virion. Then, a complimentary DNA (cDNA) strand is synthesised from the viral RNA using purified Reverse Transcriptase. In some protocols, a special RNAse enzyme is then added to nick the RNA and allow it to be degraded. The next key step occurs when a DNA polymerse and a primer generates a complimentary DNA strand just as in the PCR reaction. At the end of this reaction, a

single strand of viral RNA has been converted to a dsDNA that has the same sequence of nucleotide base. The quantitative PCR reaction can proceed as described previously.



In the light of the above known factors, we decided to look at the presence of Human Papillomavirus in patients with oral cancers and compare them to those without oral cancers.

MATERIALS

AND

METHODOLOGY

a) Study Design:

Prospective, case control study.

This study was approved by the Institution Review Board (IRB).

b) Subjects:

All patients with histologically proven Oral squamous cell carcinomas (confirmed in Pathology Department, CMC Vellore) who came to General Surgery I OutPatient department were chosen for this study.

c) Sample size:

Th**e** samplesize was calculated based on the existing data. The prevalence among the cases (exposed) was 40% and the prevalence among the controls (the non-exposed) was 10%. When the power of the study was taken as 80% and the significance was calculated to be 5% (p value : 0.05) the sample size came to 76 with 38 in each arm. Hence the number of cases and controls was decided to be 40 each. This was calculated with the help of a statistician.

d) Inclusion criteria for cases:

- Histologically confirmed oral squamous cell carcinoma
- Any stage of oral squamous cell carcinoma
- Any gender

e) Exclusion criteria:

- History of previous radiation or chemotherapy
- Presence of any premalignant lesions.

f) Case sample:

Mucosal scrapings from the oral cavity which was taken using a small brush.

g) Control**s:**

Age and gender matched patients who come to General Surgery OPD for treatment of other conditions.

h) Informed consent:

Informed consent was taken from all patients enrolled in the study. The consent form is attached as Appendix

i) Methodology:

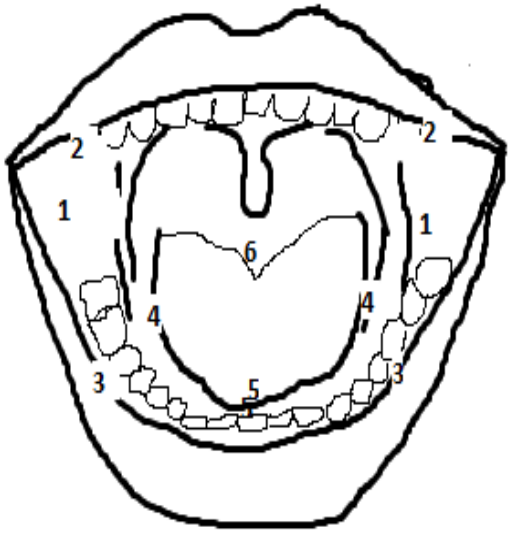
Sample collection and processing of sample:

The patients with oral squamous cell cancers were identified as cases and oral mucosal scrapings were done. The specimen were collected by the principal investigator using the same method for all patients to prevent bias in the sample collection. The cases were identified, the

details of the study were explained and informed consent was obtained. Then the patient was asked to open his/her mouth wide. Scrapings were taken from the oral mucosa by gentle sweeps along the mucosa in the following areas

1. The right and left buccal mucosa
2. The right and left upper gingivobuccal sulcus
3. The right and left lower gingivobuccal sulcus
4. The right and left lateral borders of the tongue.
5. The floor of the mouth
6. The posterior one third of the tongue, which was done last to prevent gag and discomfort to the patient.
7. In patients with oral lesions gentle sweeping of the tumour was also done

In each of these sites ten strokes of the brush was made to assure the adequacy of the sample collected.



The samples were then mixed with the transport media which was a prepacked product from Hybribio. This specimen was placed in an ice box and was transported to the Department of Virology. In the department these samples were stored at a temperature of $4^{o}C$ till they could be processed for the RT PCR.

RESULTS

The following tables represent the analysis of the data that was collected.

Figure 1:

The gender distribution of the cases and controls. The cases were matched with the controls based on their gender.

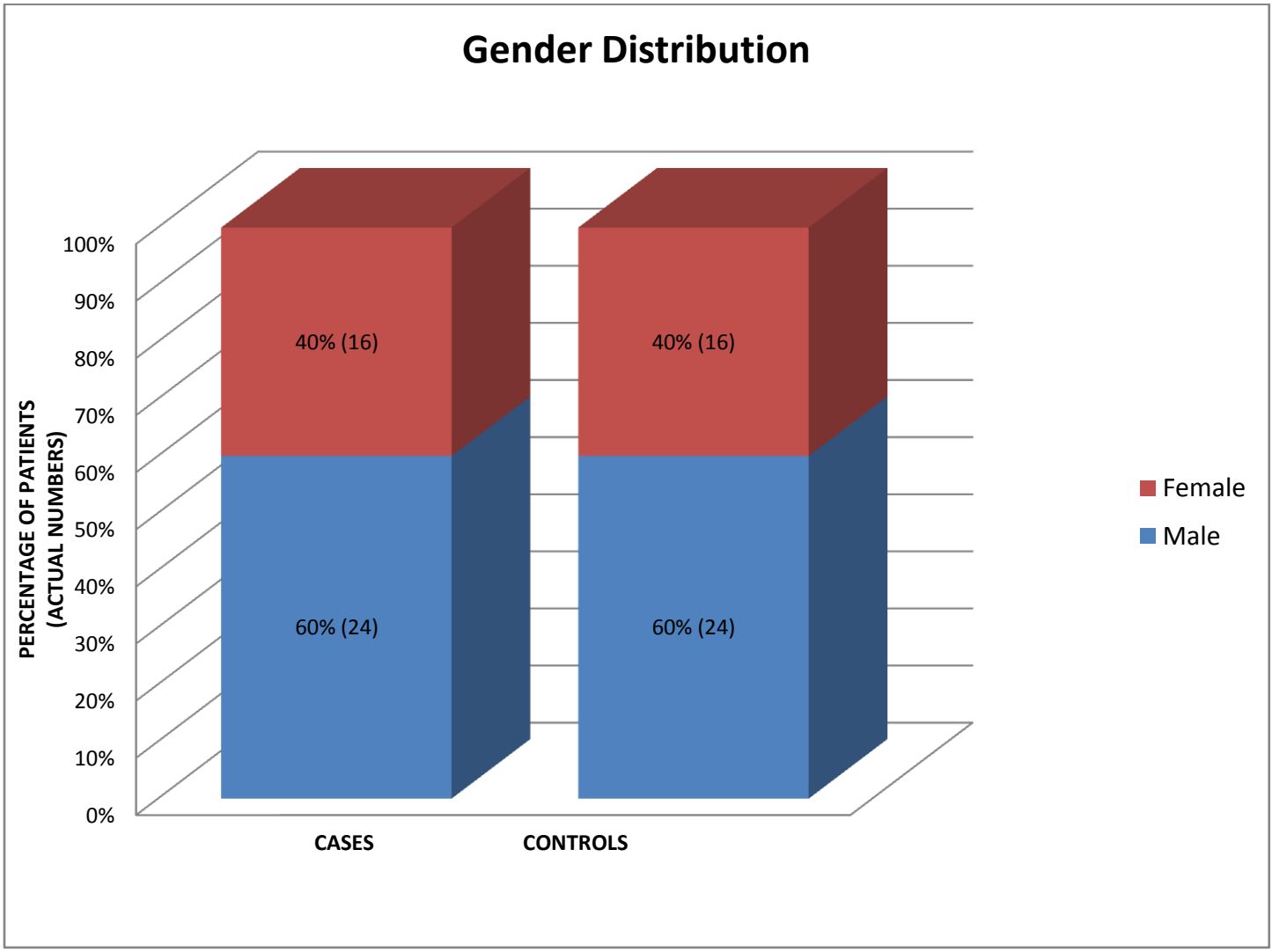


Figure 2:

The following figure shows the geographic representation of the patients who presented with oral cancers to the General Surgery OPD

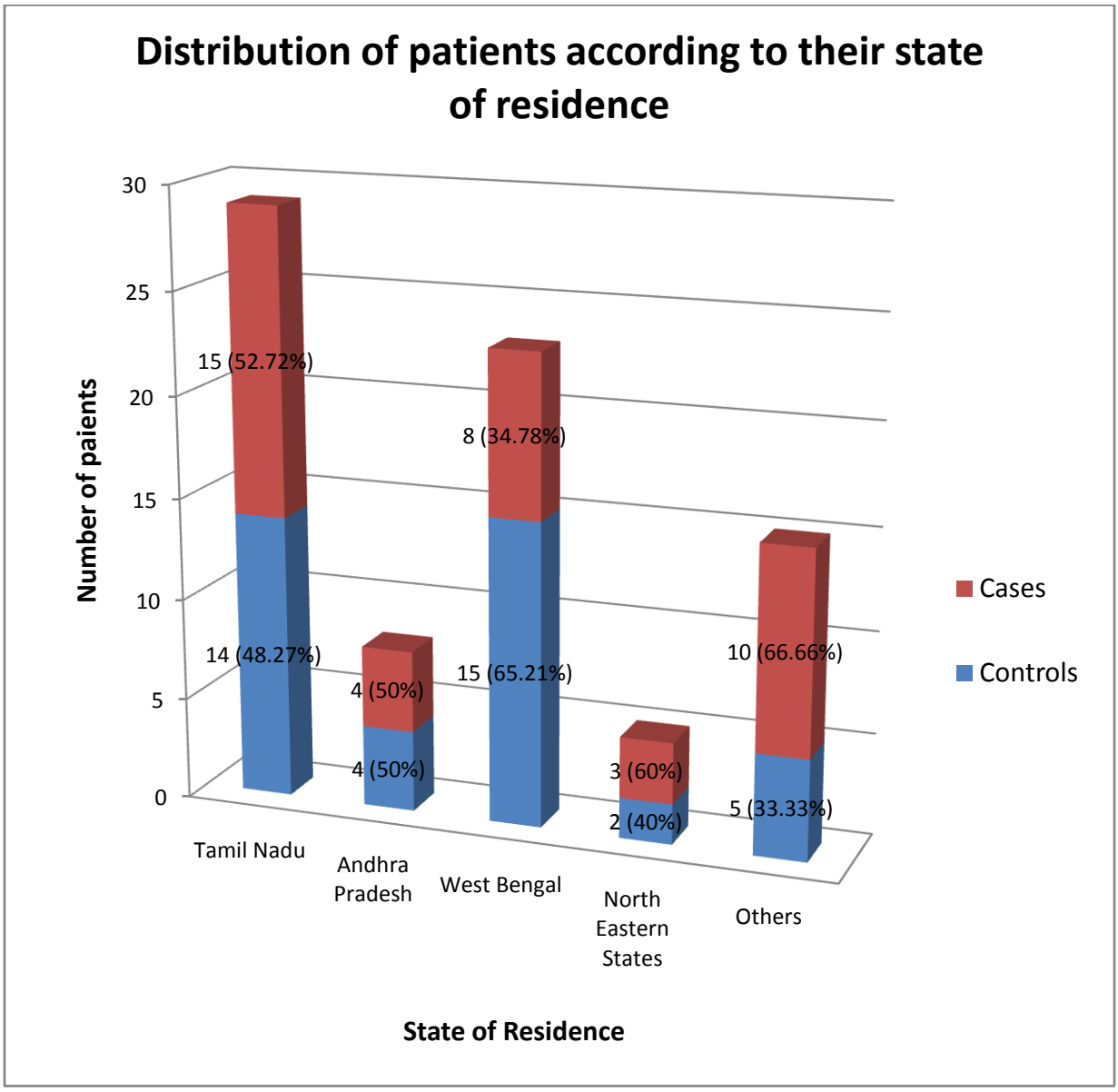
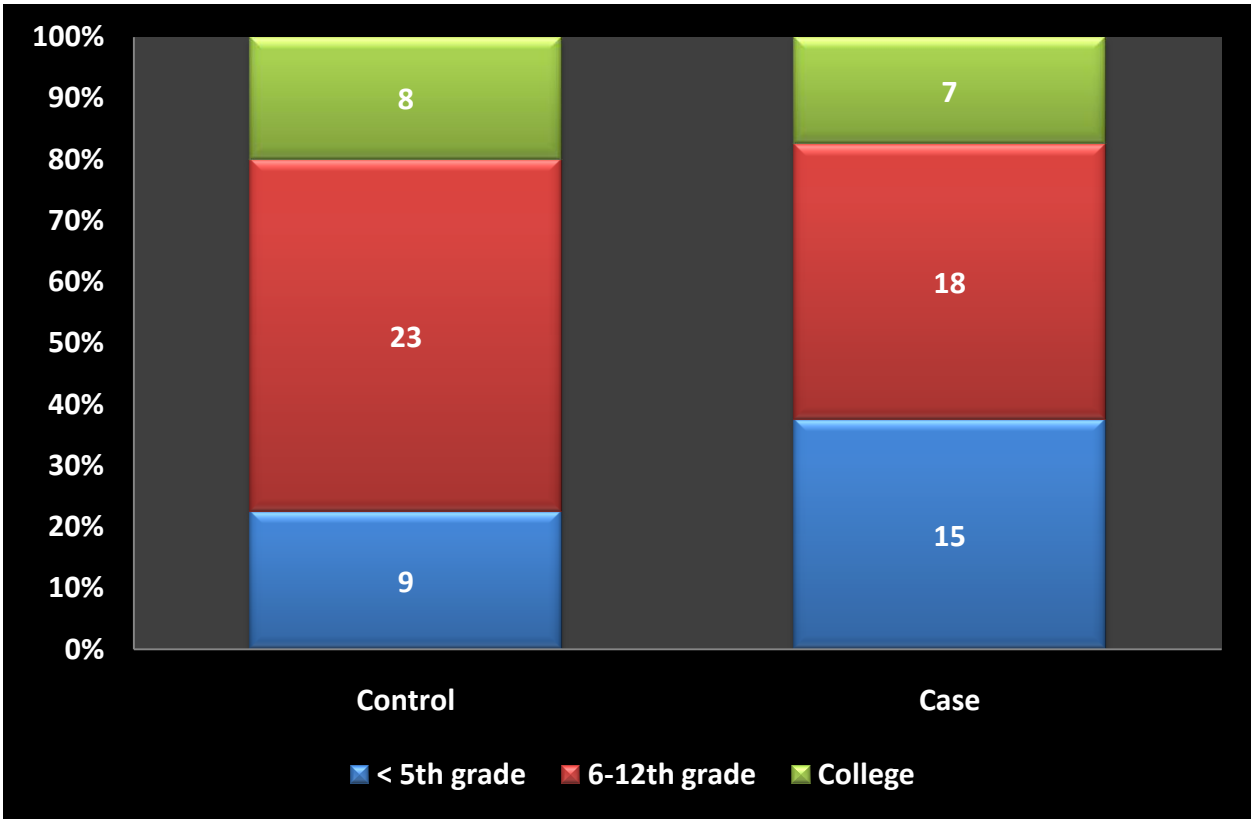


Figure 3:

The education level of the cases and controls were as follows.



Further breakdown of education level in the cases and controls was as follows

Figure 4:

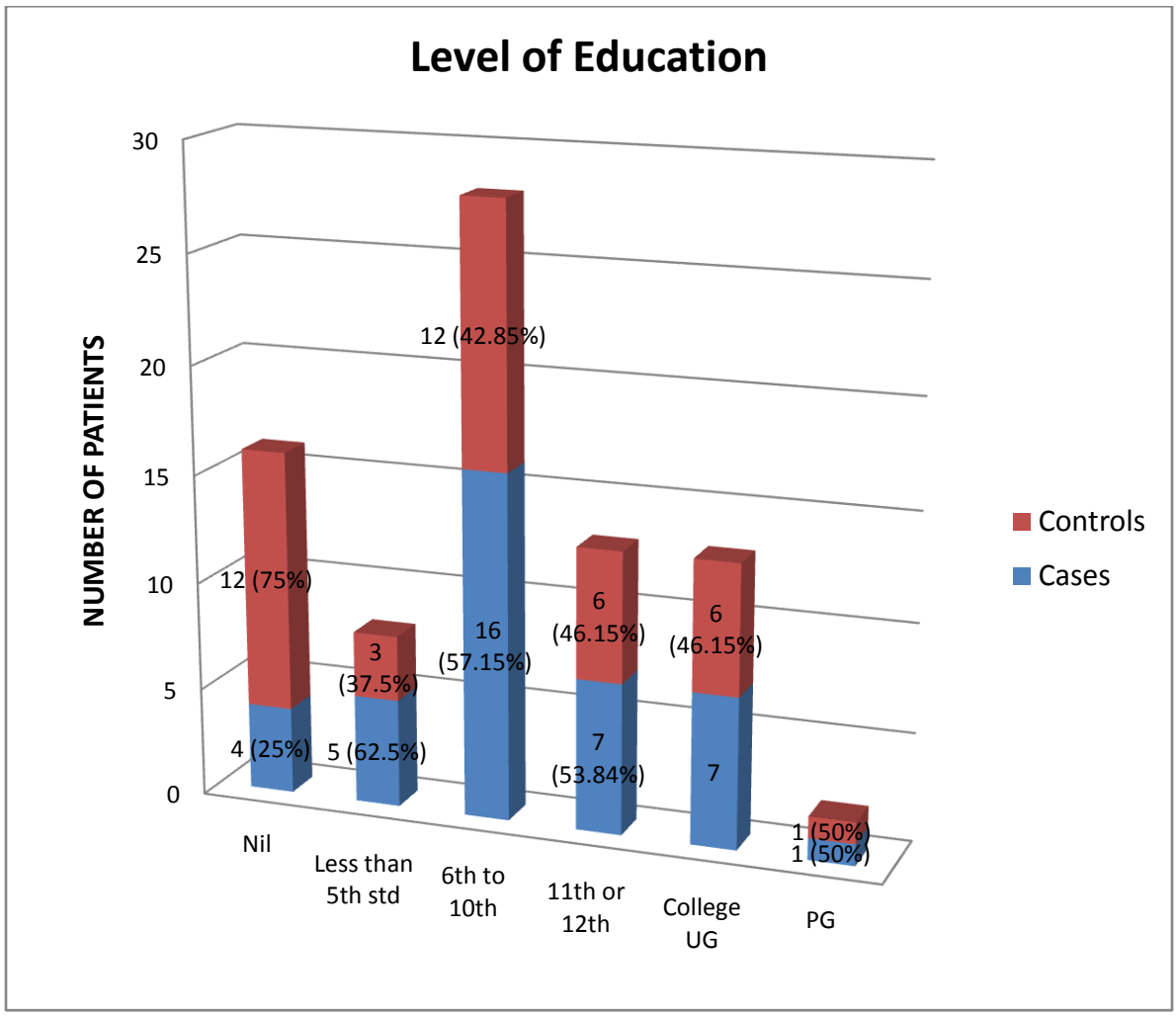
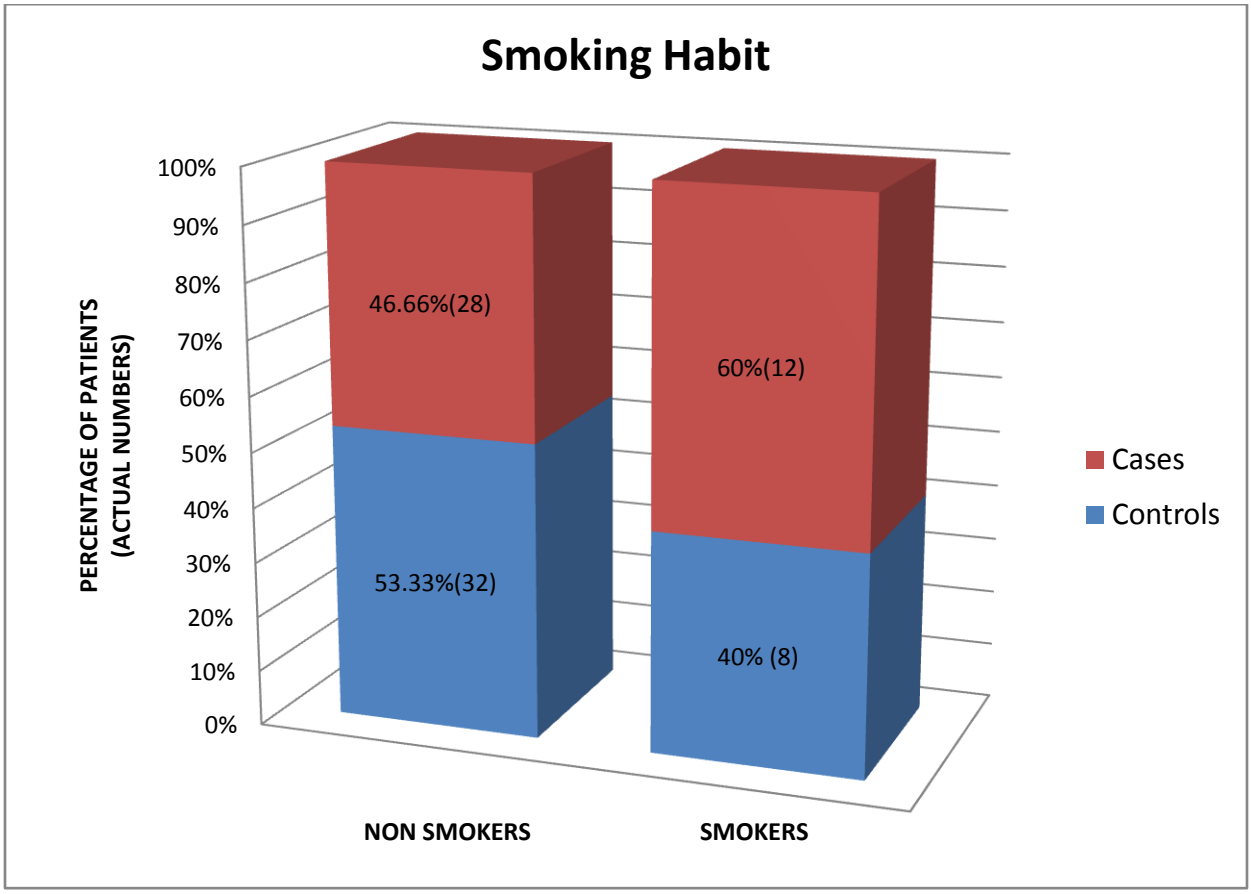


Figure 5:

The following tables show the percentage of people who smoke among the cases and controls. Smoking is considered one of the common causative factors of Oral Squamous cell carcinomas.



The smoking habits were further analysed with regard to the number of years of smoking, the frequency of smoking and the type of tobacco used. The findings were as follows.

Figure 6: Frequency of smoking:

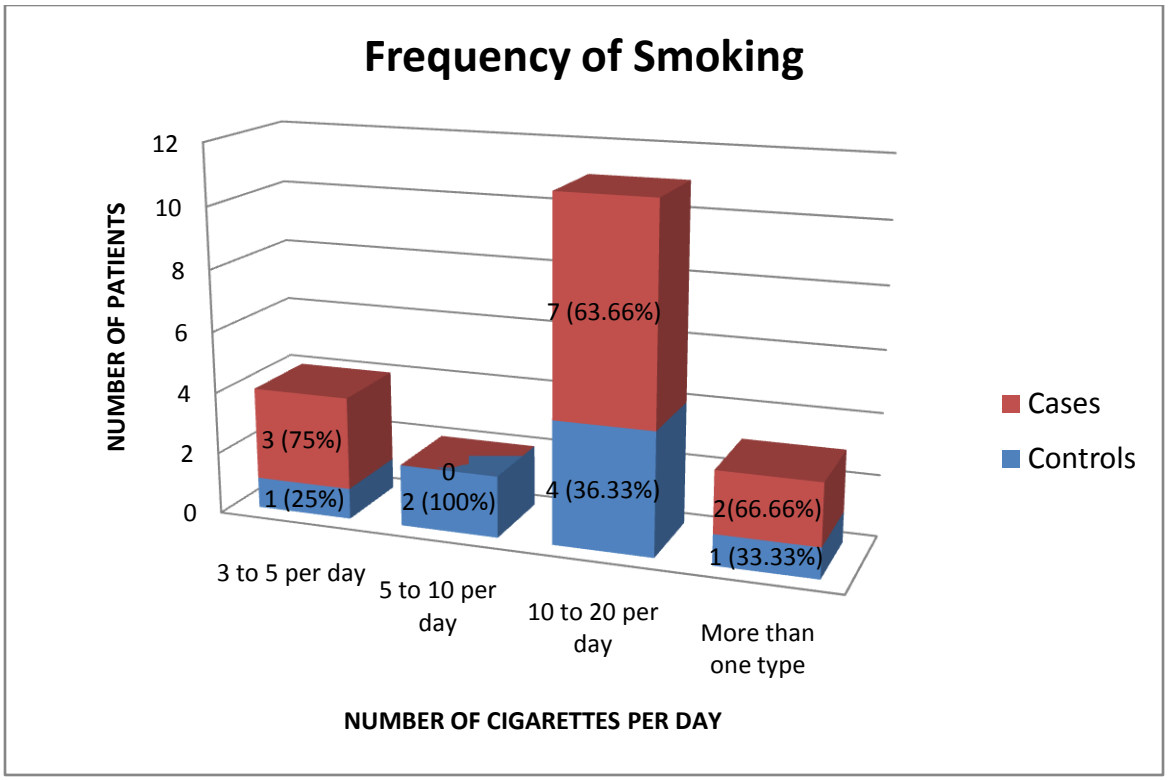


Figure 7 : Shows the various methods of smoking tobacco

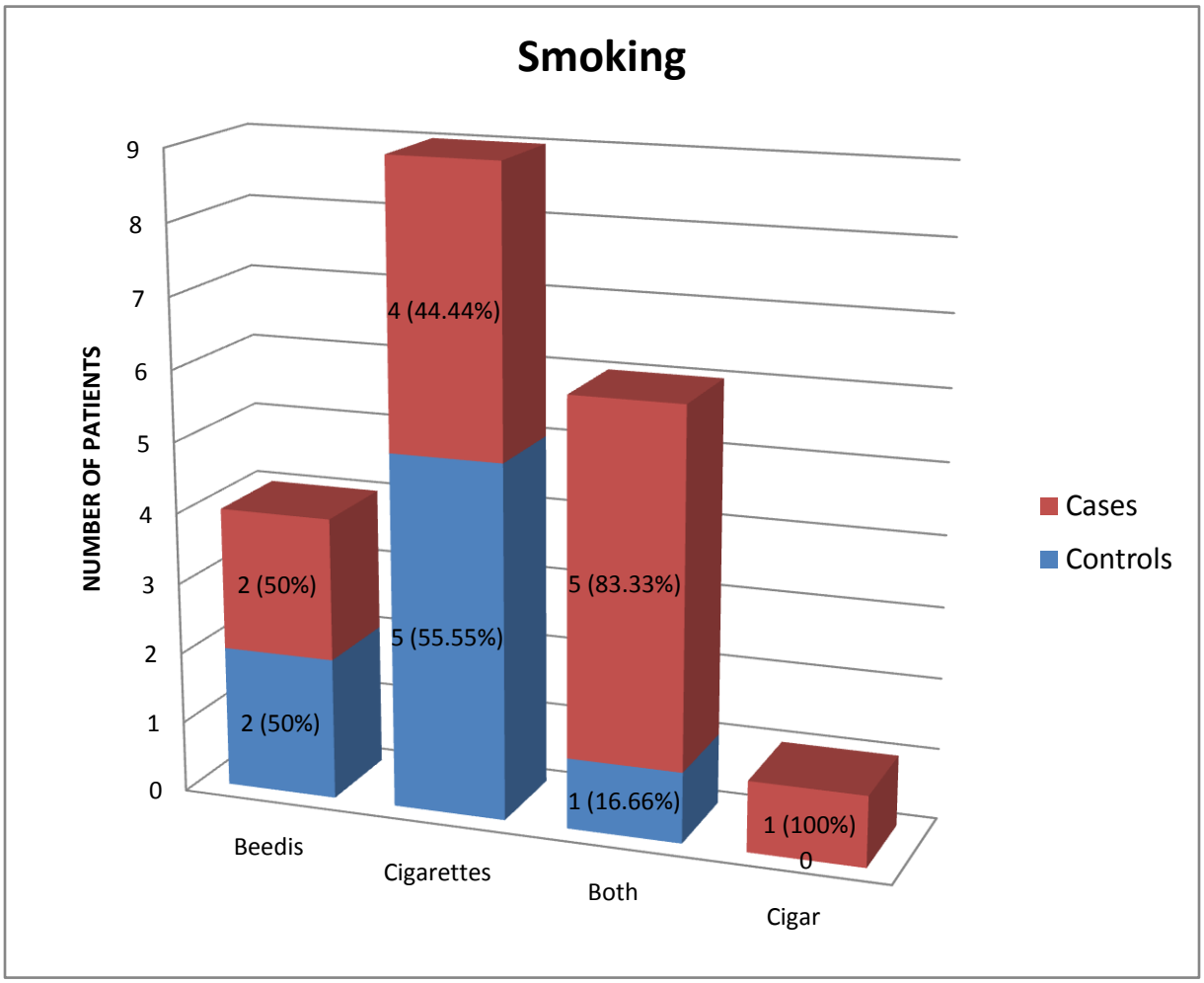


Figure 8:

This indicates the number of years the patient had been smoking.

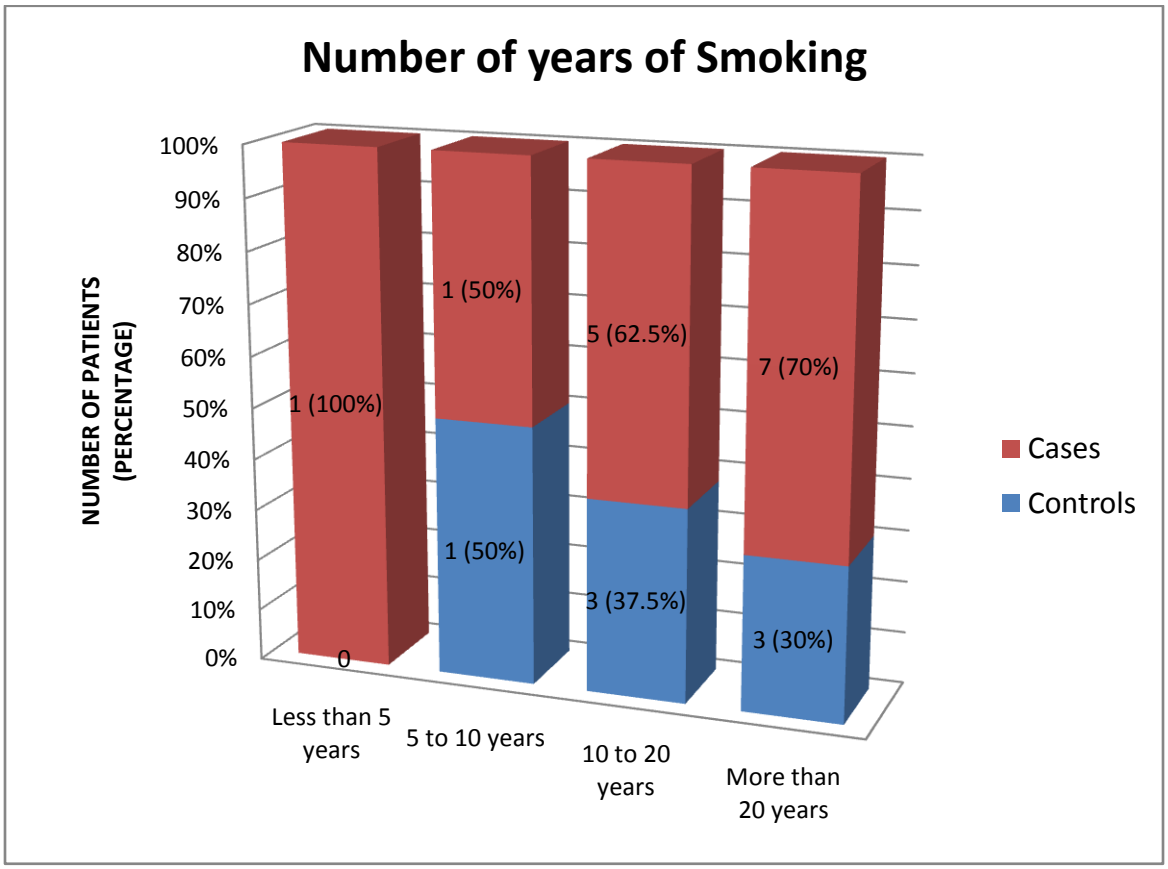


Figure 9:

This graph indicates the number of people who chew tobacco in the cases and the Control group.

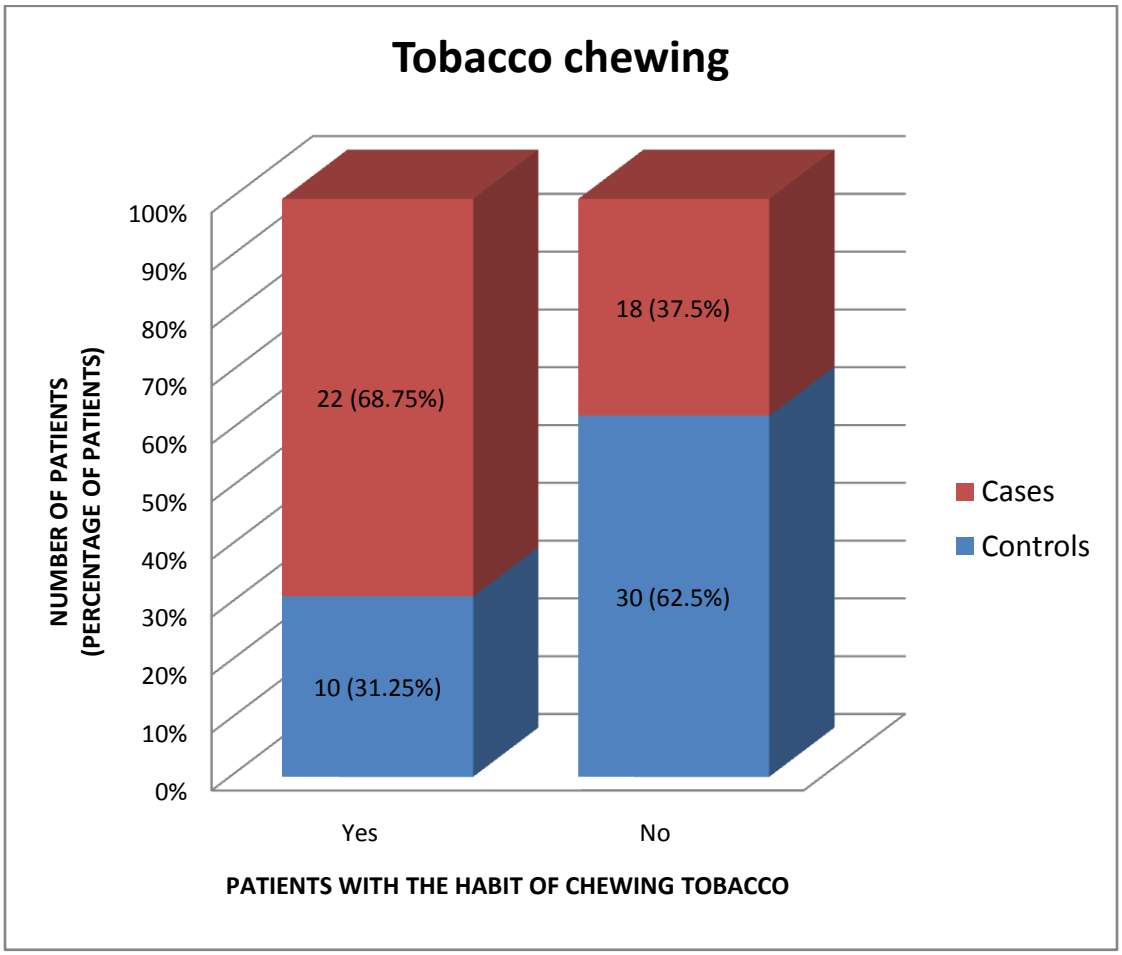


Figure 10:

The following graph indicates the frequency of chewing tobacco among the cases and controls.

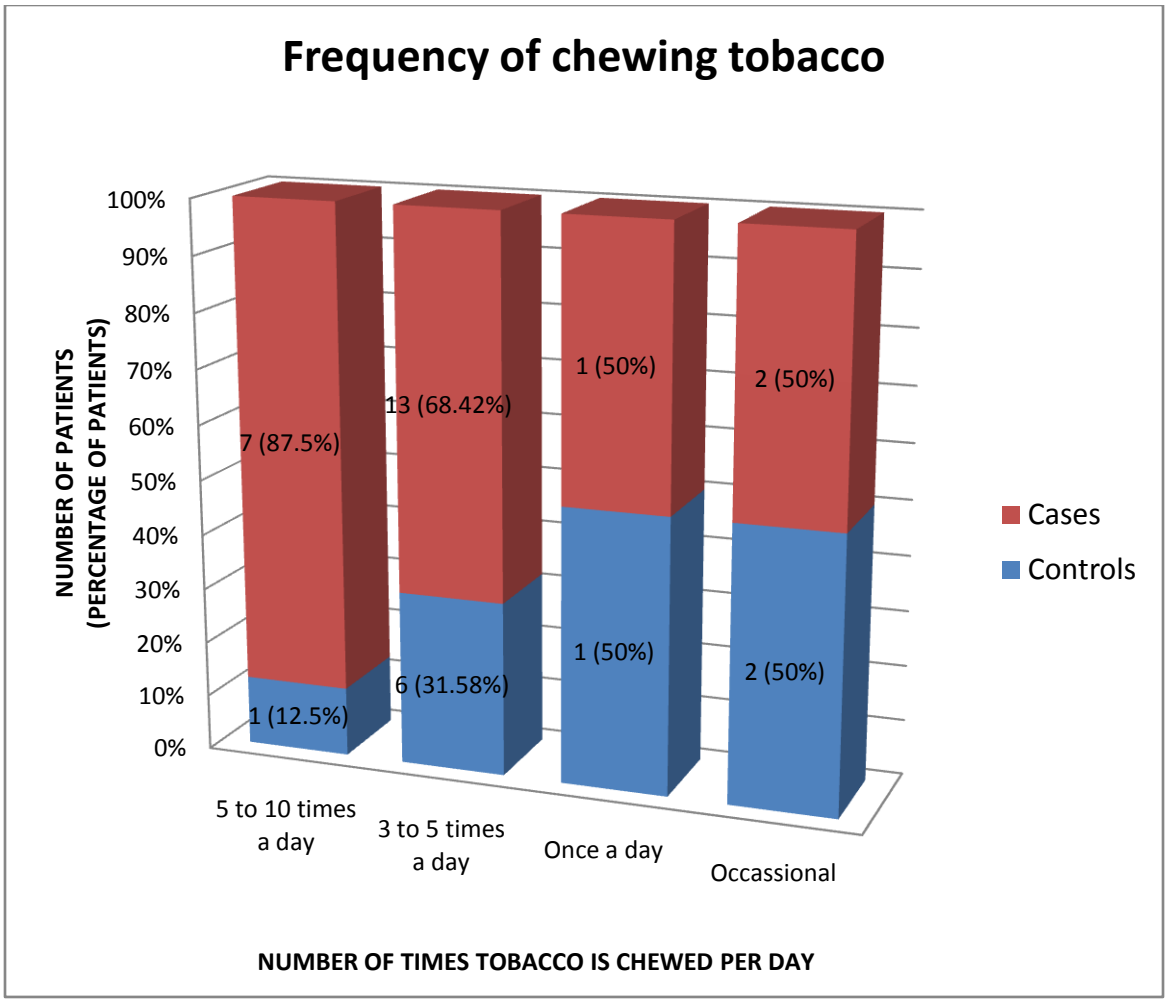


Figure 11:

The following table breaks down the different types of tobacco that was chewed among the cases and controls.

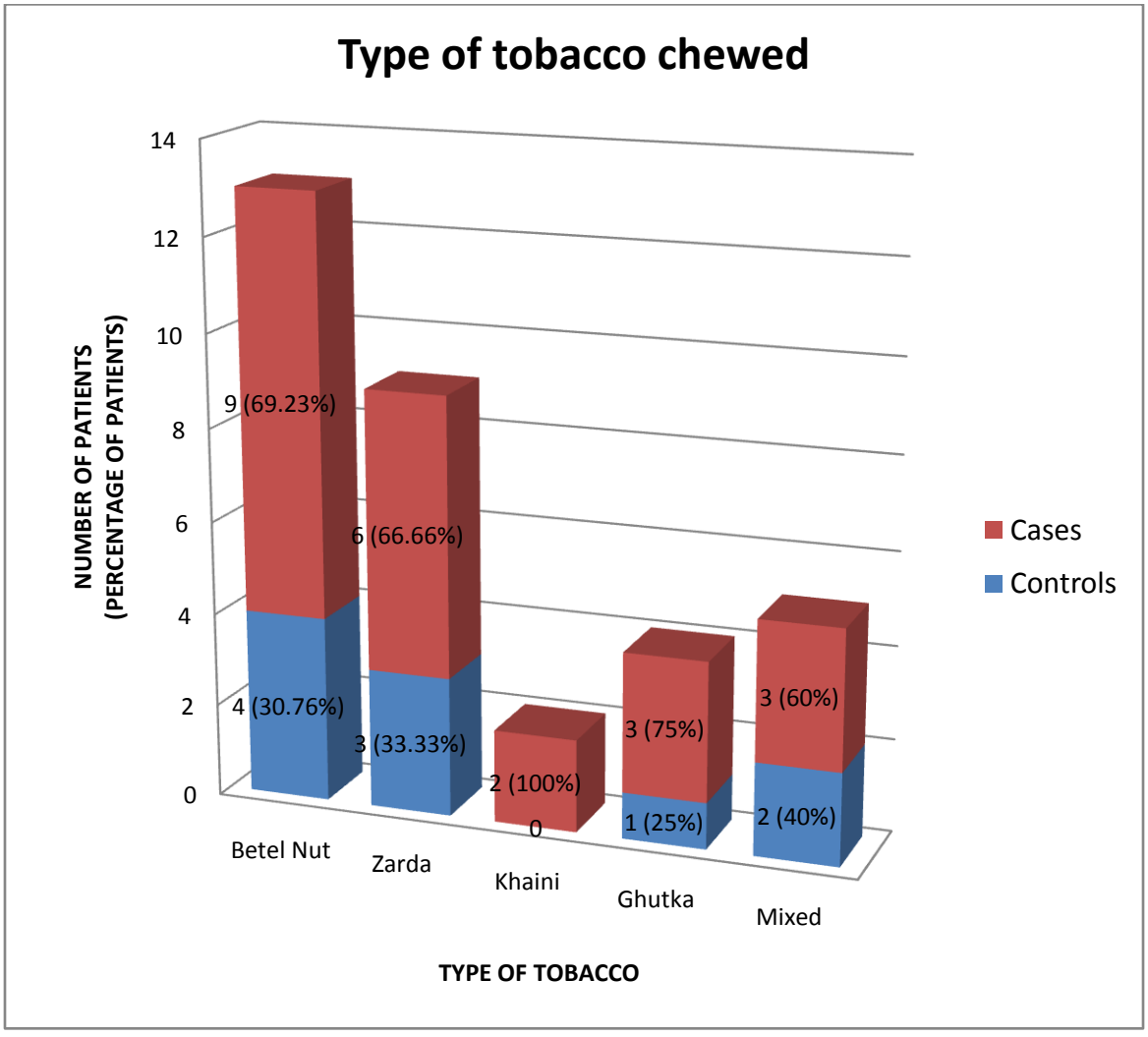


Figure 12:

The following table represents the number of years the patients in both the cases and the controls group have chewed tobacco.

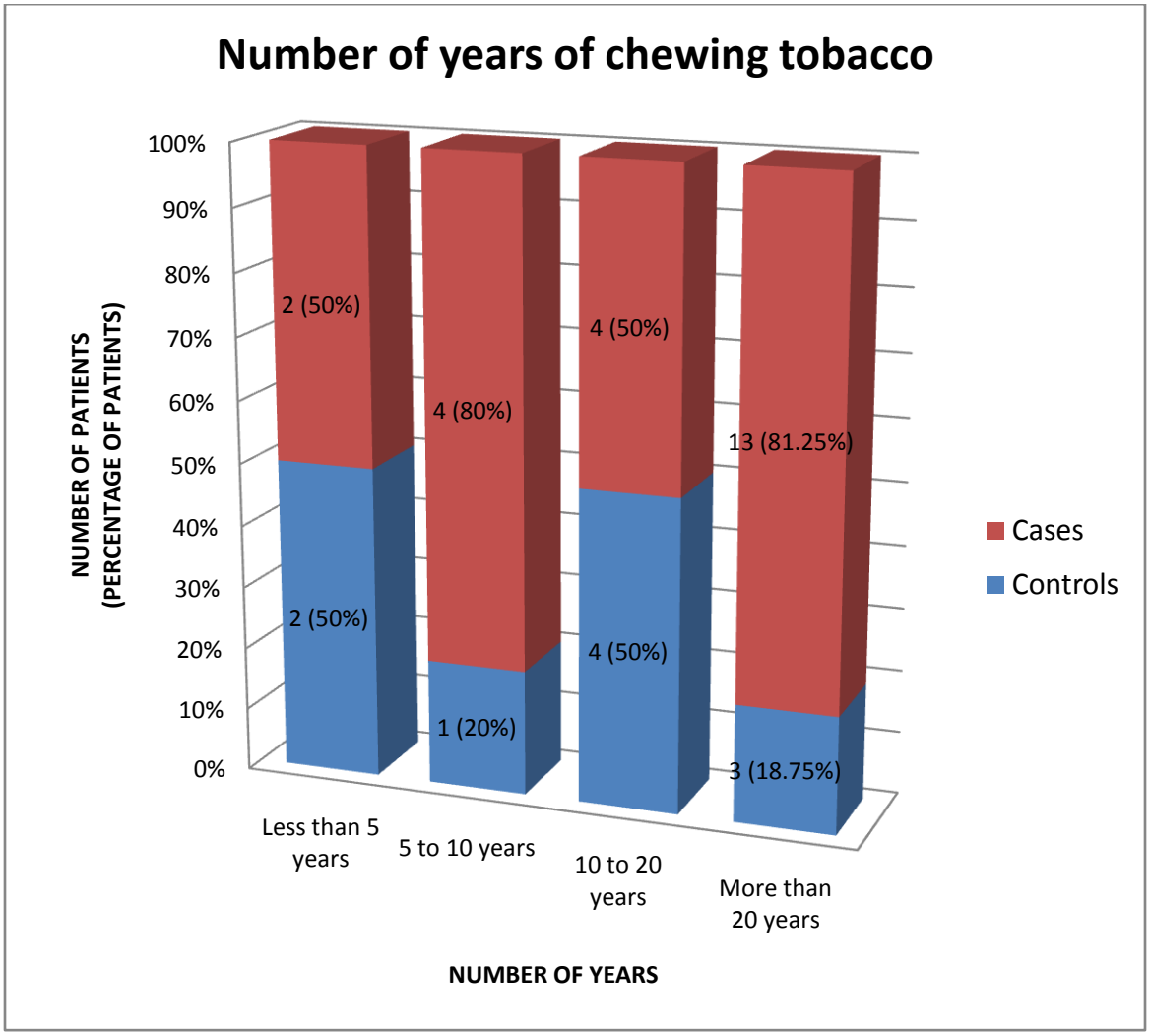
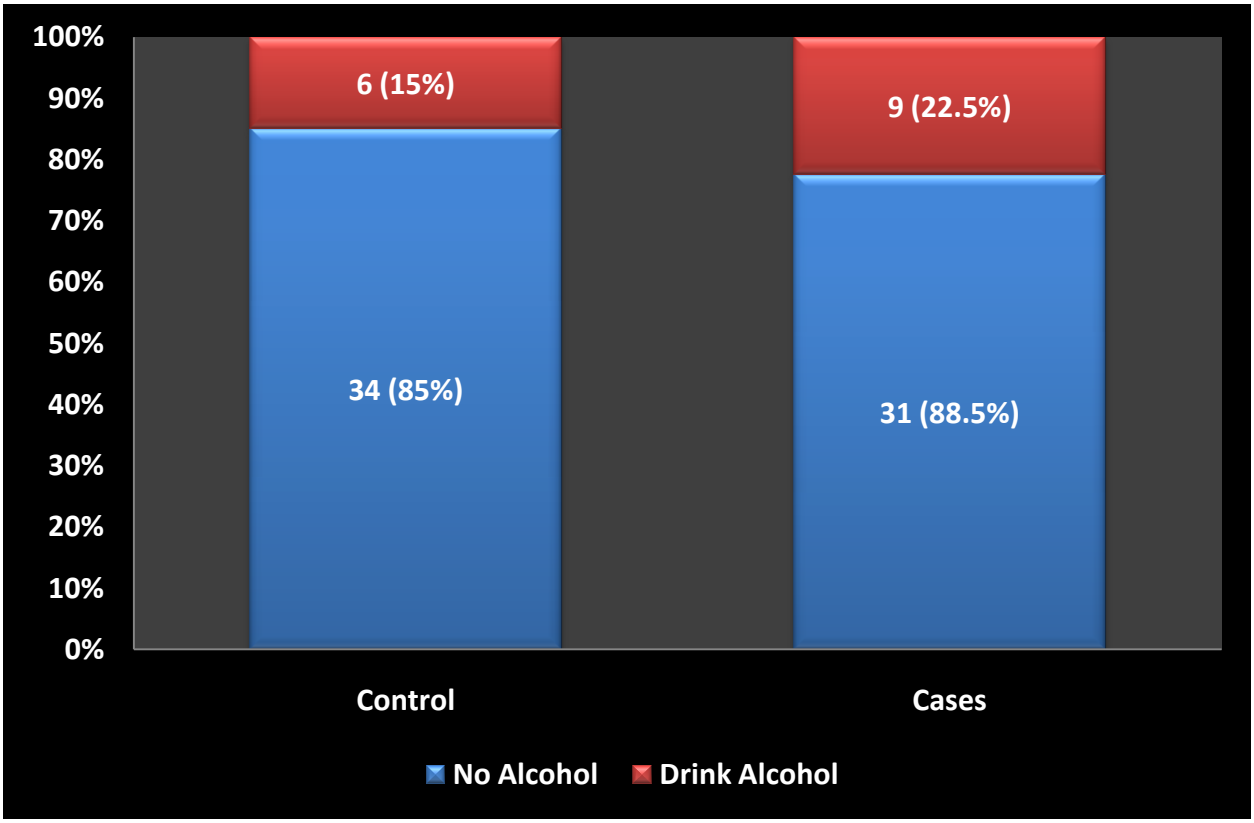


Figure 13:

The following graph shows the percentage of people who consumed alcohol in the cases and the control group.



The following tables discuss the type of alcohol consumed in both the cases and the control group along with the frequency of consumption and the number of years of consumption of alcohol.

Figure 14:

The following table indicates the number of years of alcohol consumption.

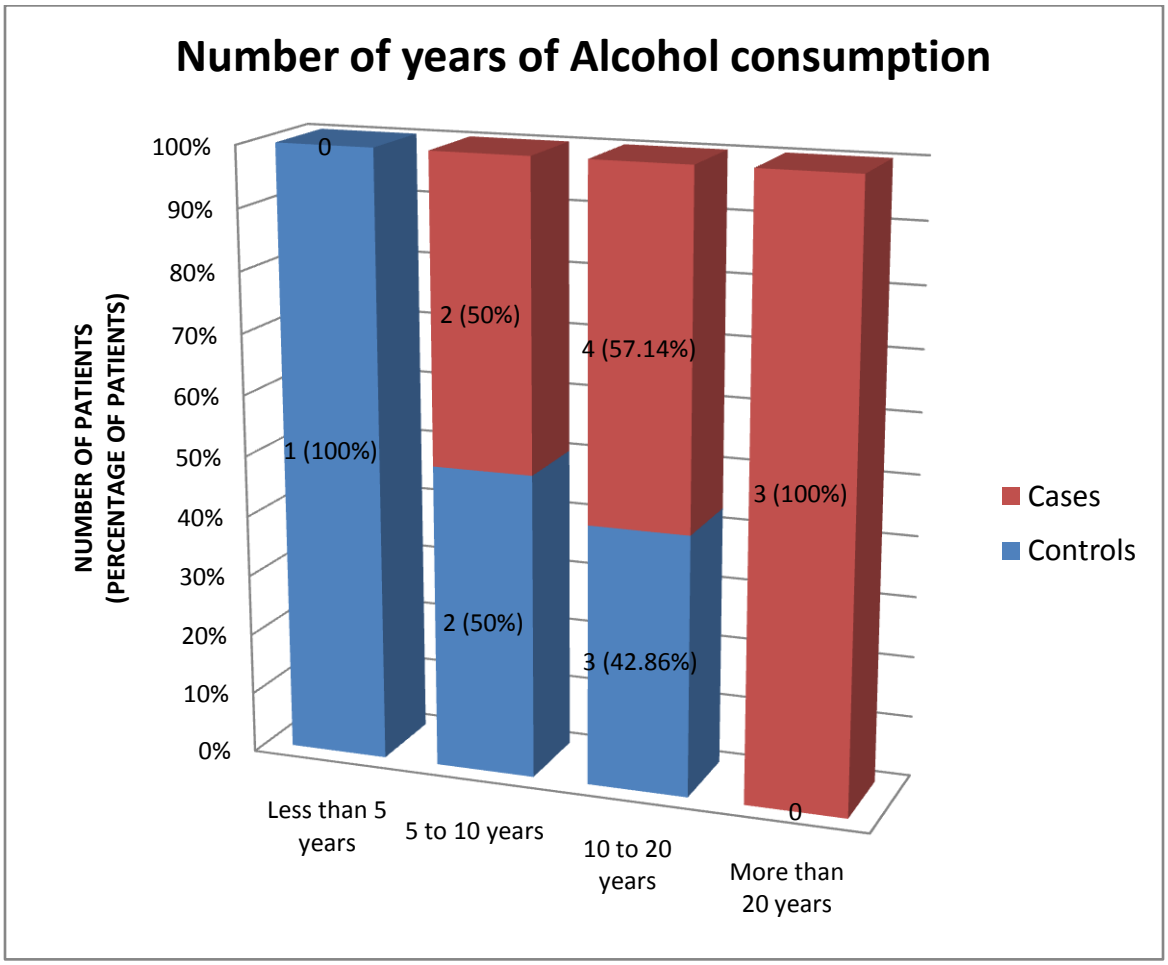


Figure 15:

The different types of alcohol consumed was also compared among the cases and the controls to see whether there was a particular type of alcohol which was causative in oral cancers.

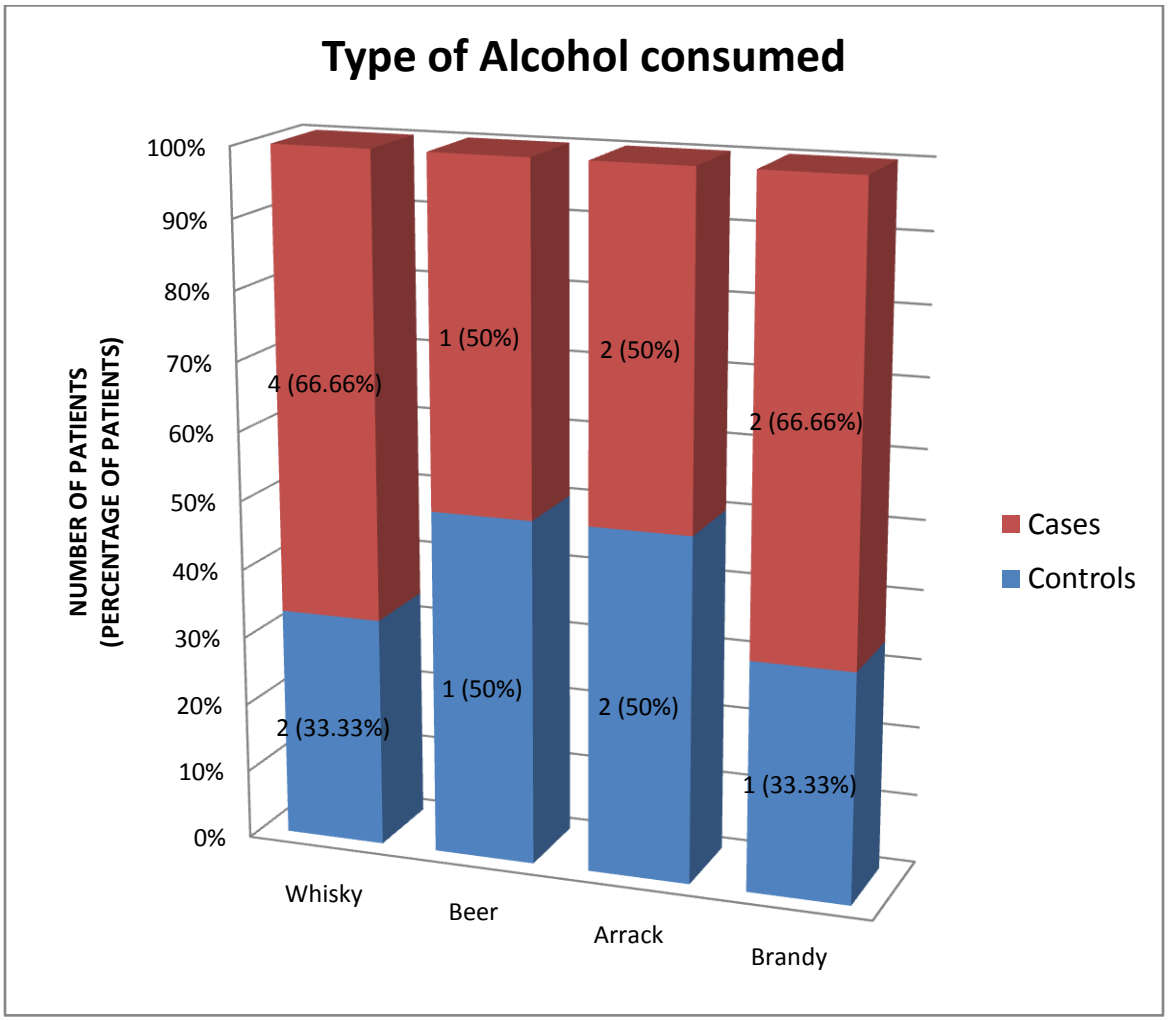
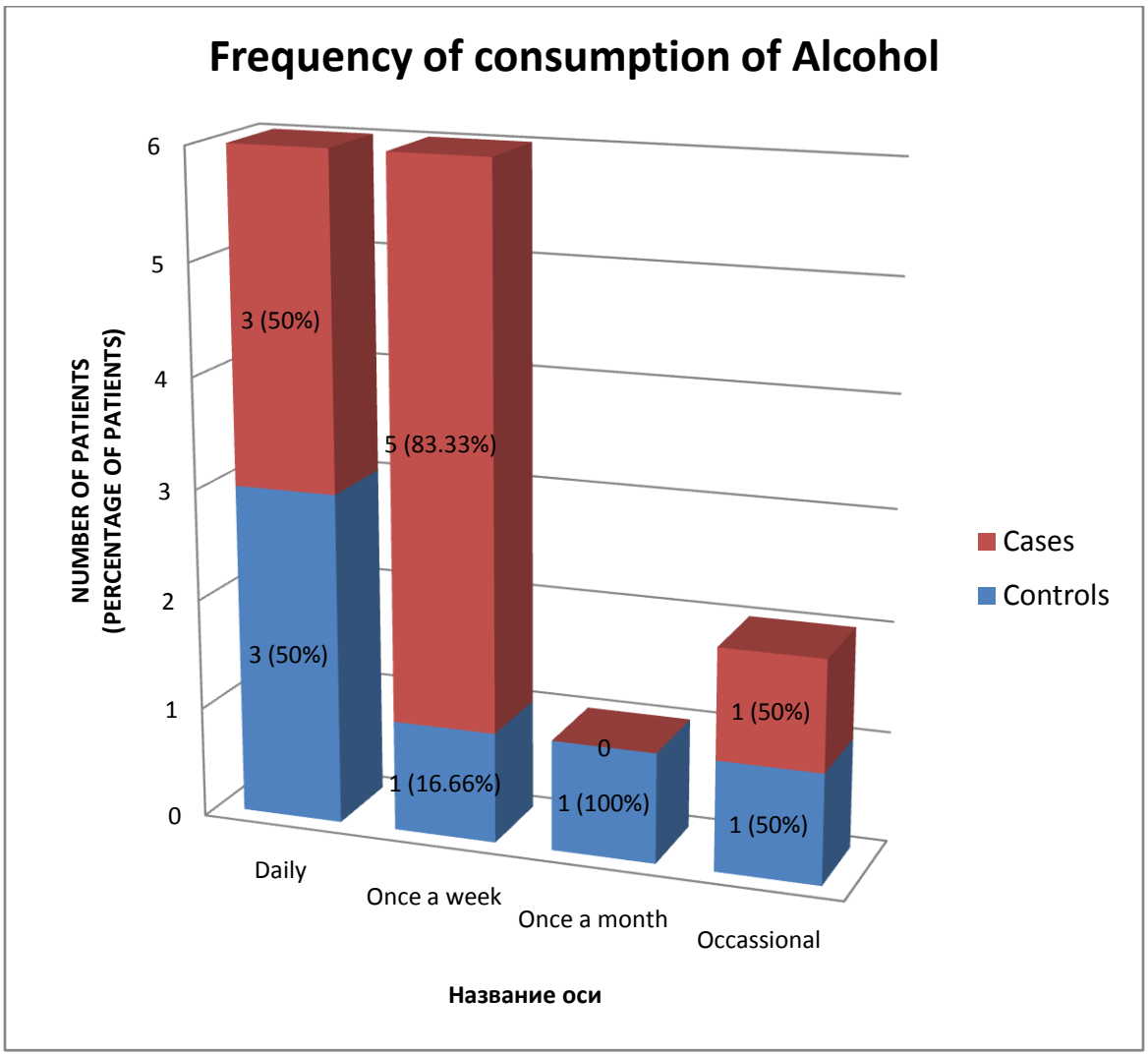


Figure 16:

The following table indicates the frequency of consuming alcohol.



The following tables discuss the other co-morbid conditions that can cause oral cancers. The frequency of dental visits and other dental problems are compared with the presence or absence of oral cancers.

Figure 17:

Habit of brushing teeth:

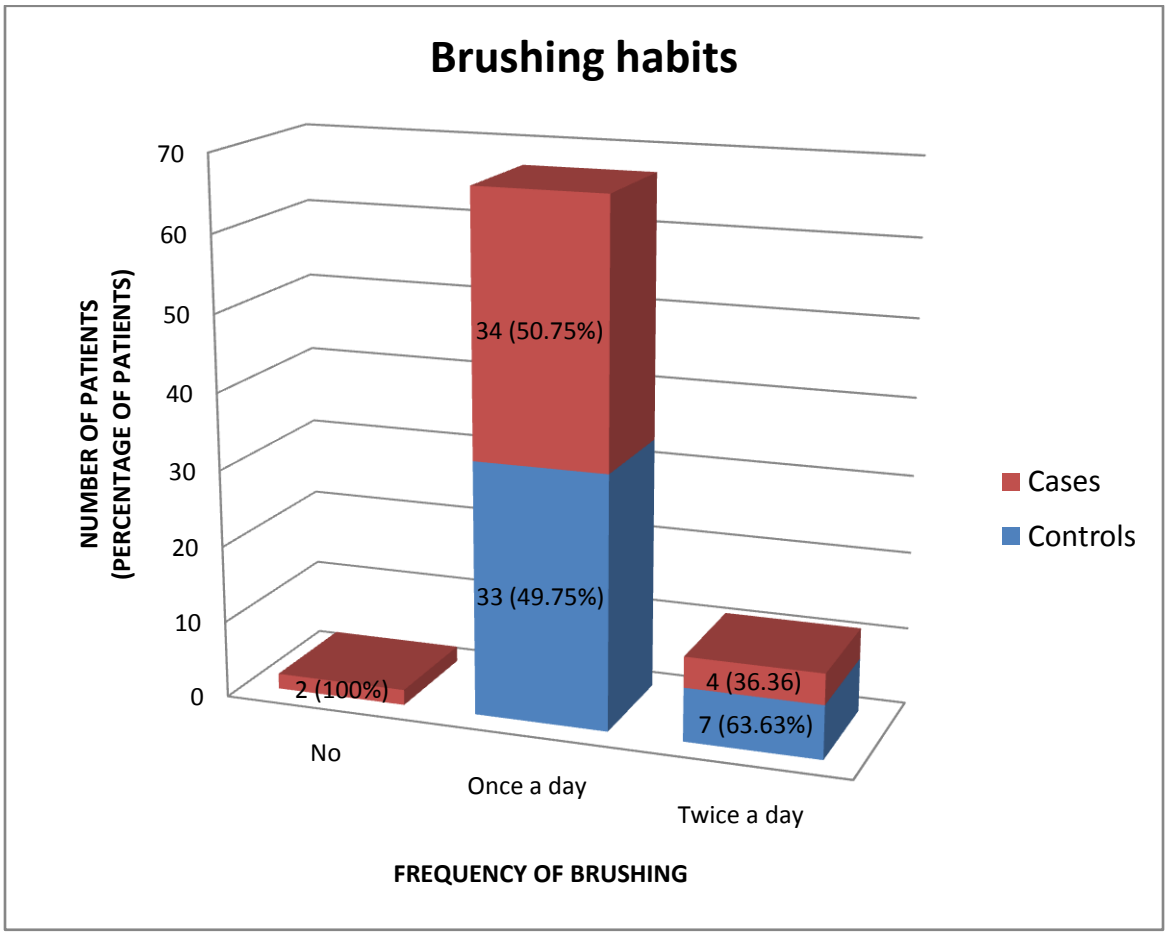


Figure 18:

The number of dental visits the patients had before the diagnosis of oral cancer was analysed.

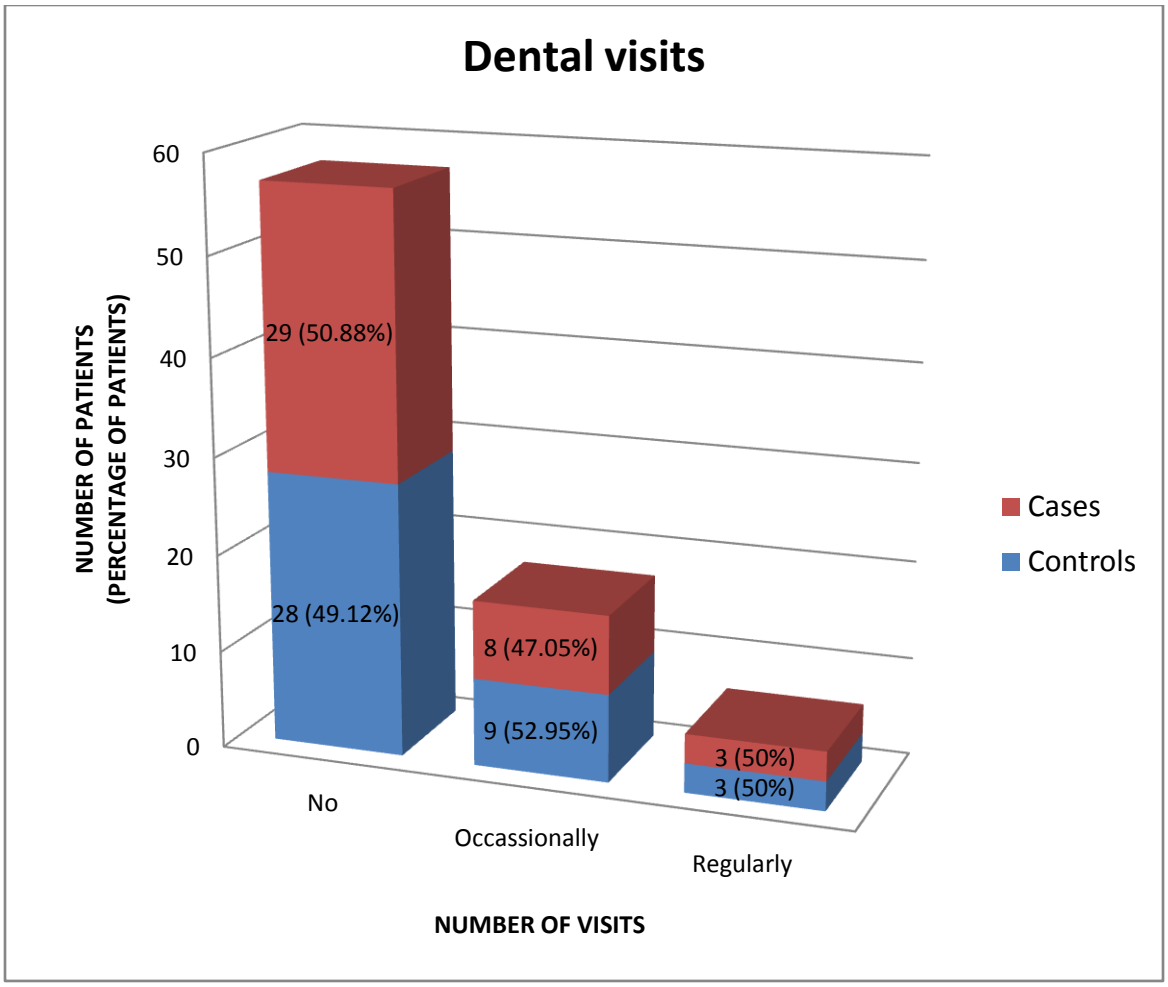


Figure 19:

Dental problems versus oral cancers.

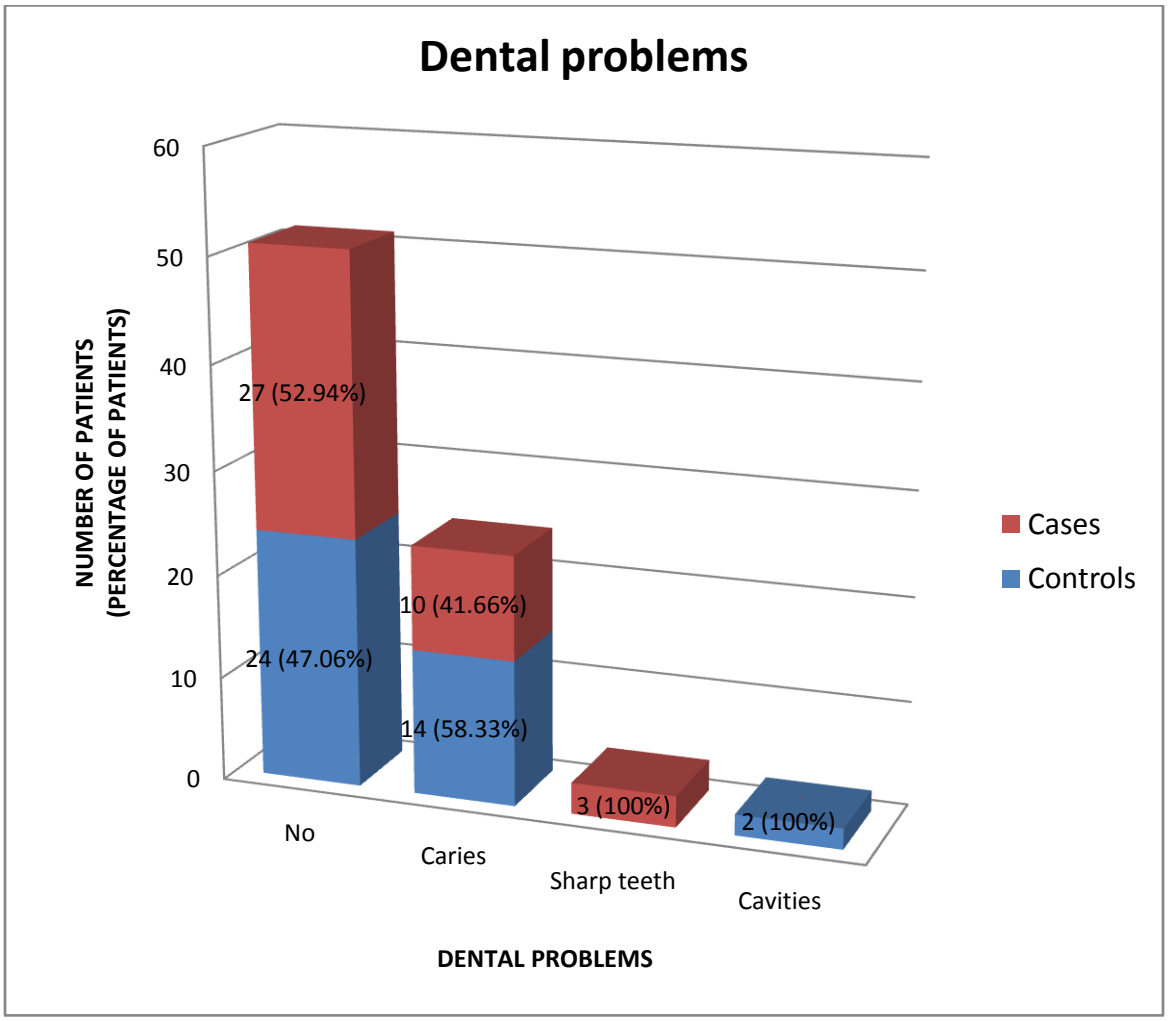
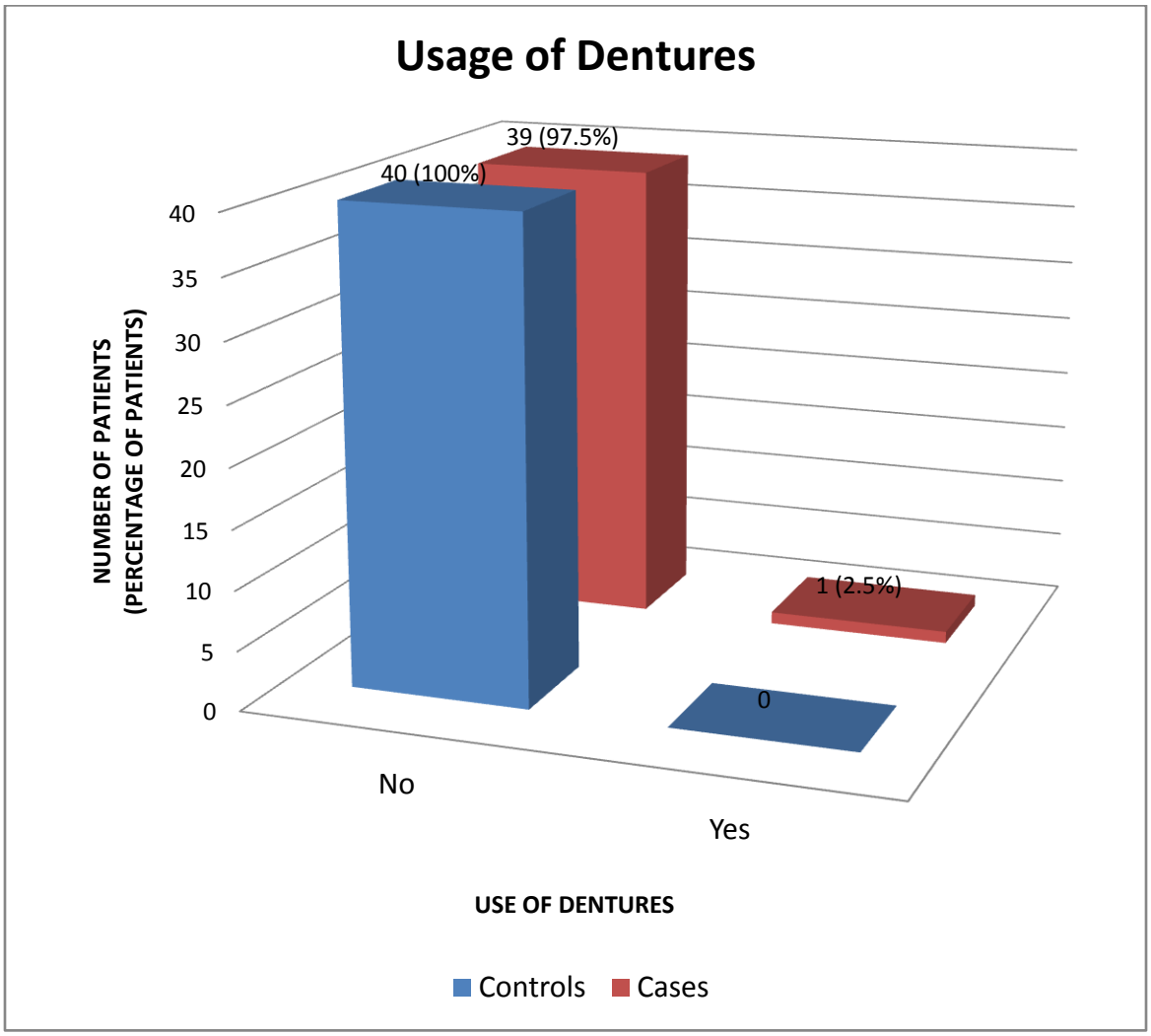


Figure 20:

The following table indicates the number of patients who were using dentures.



The next set of tables discuss the presence of co-morbid conditions like diabetes and hypertension which have been found to have a bearing on the patient's general condition which can predispose him or her to malignancies. Diabetes Mellitus is considered one of the immunocompromised states which can lead to the formation of malignancies.

Figure 21:

Figure comparing the presence or absence of oral squamous cell carcinoma against diabetes.

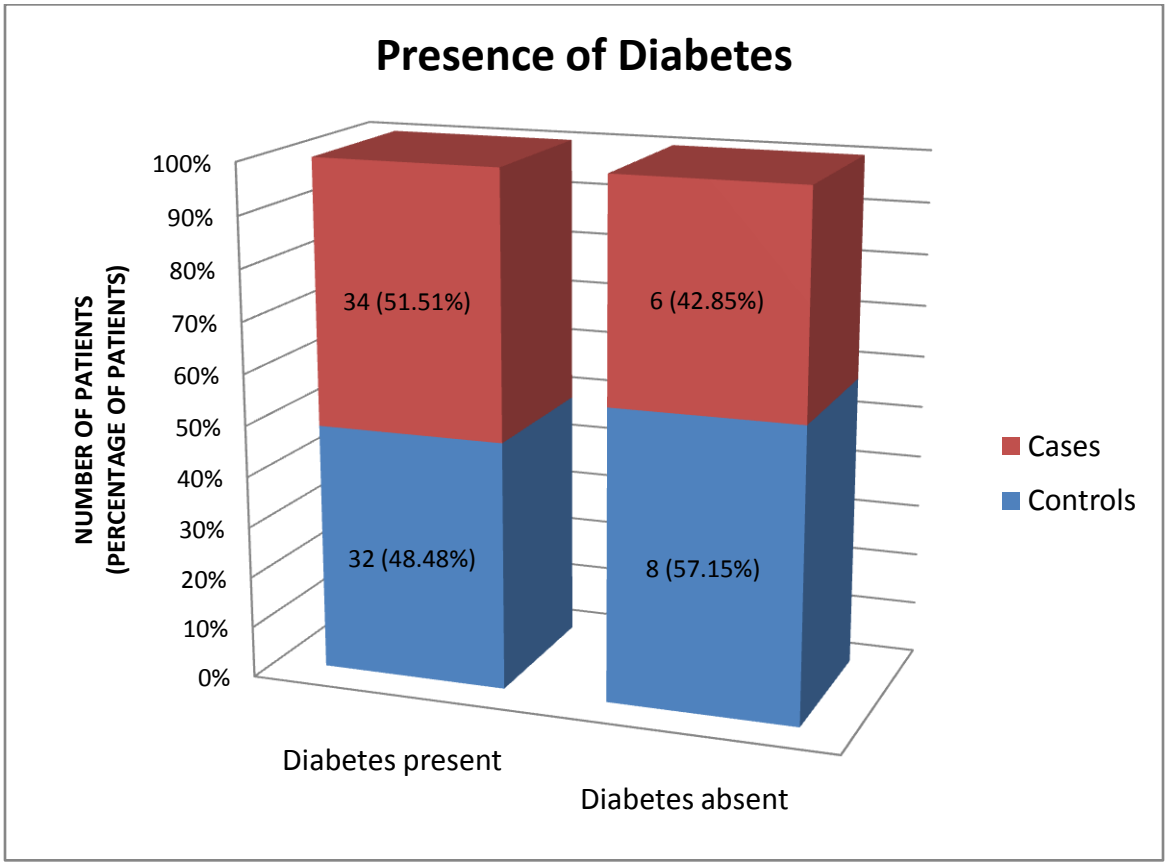


Figure 22:

The number of years of diabetes compared with the presence or the absence of oral cancers.

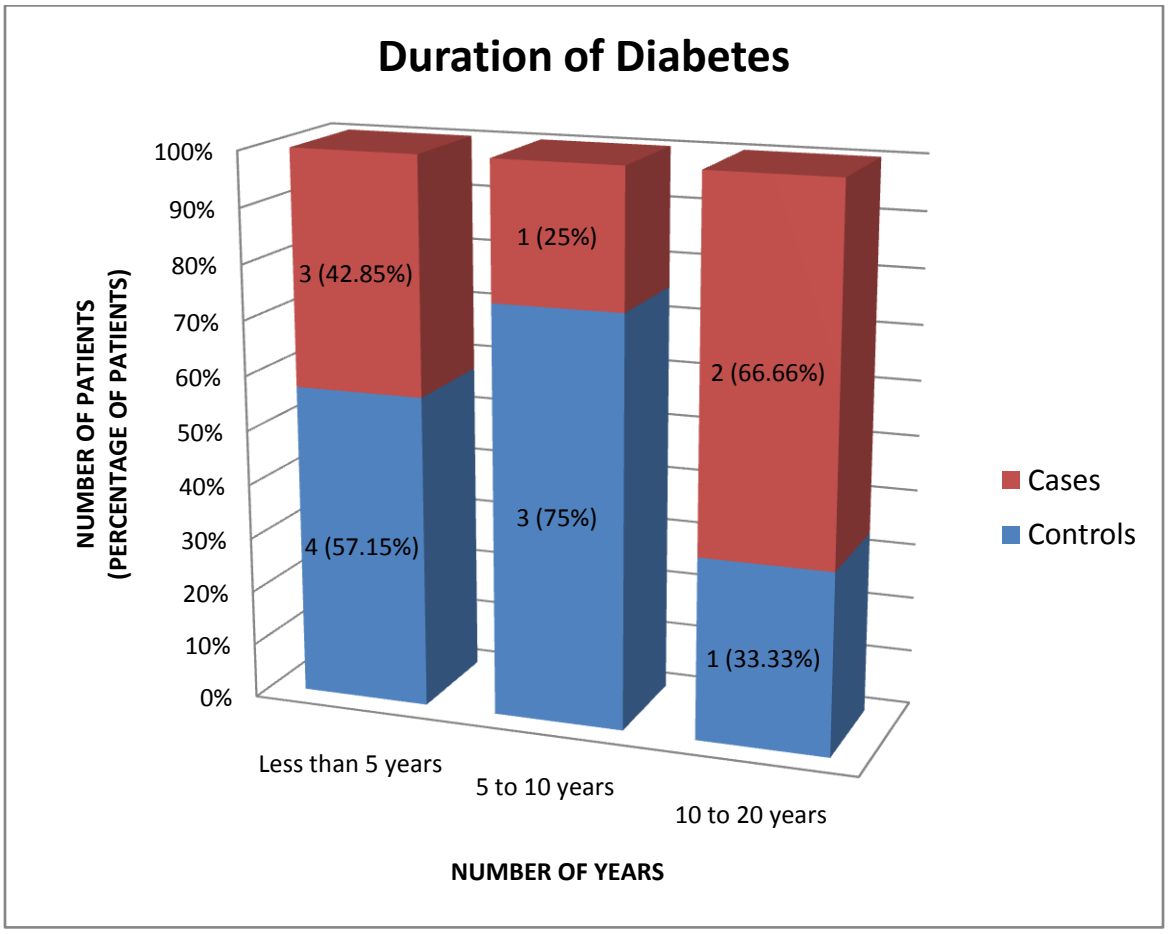


Figure 23:

The presence of Hypertension in those with and without oral cancers

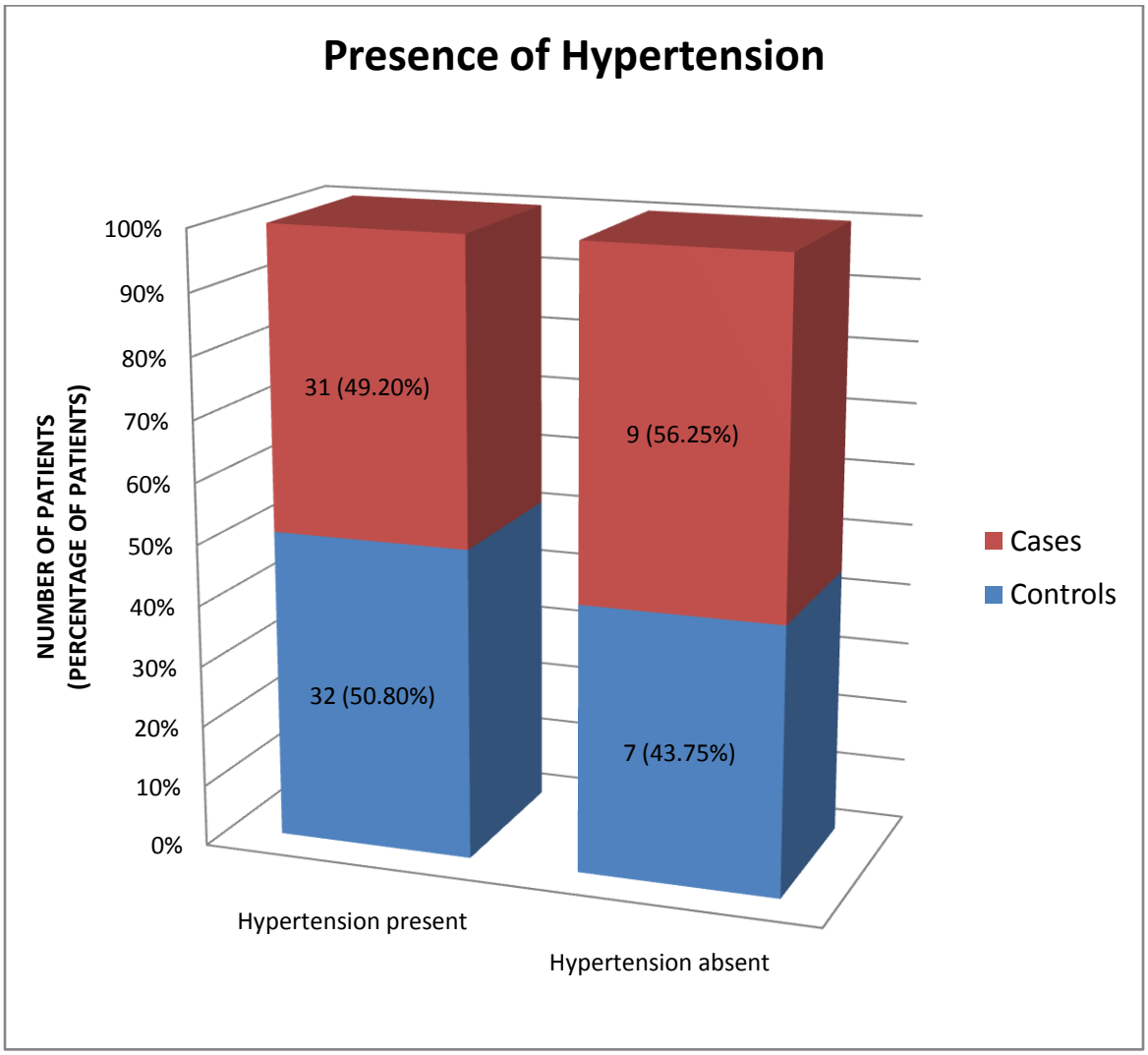
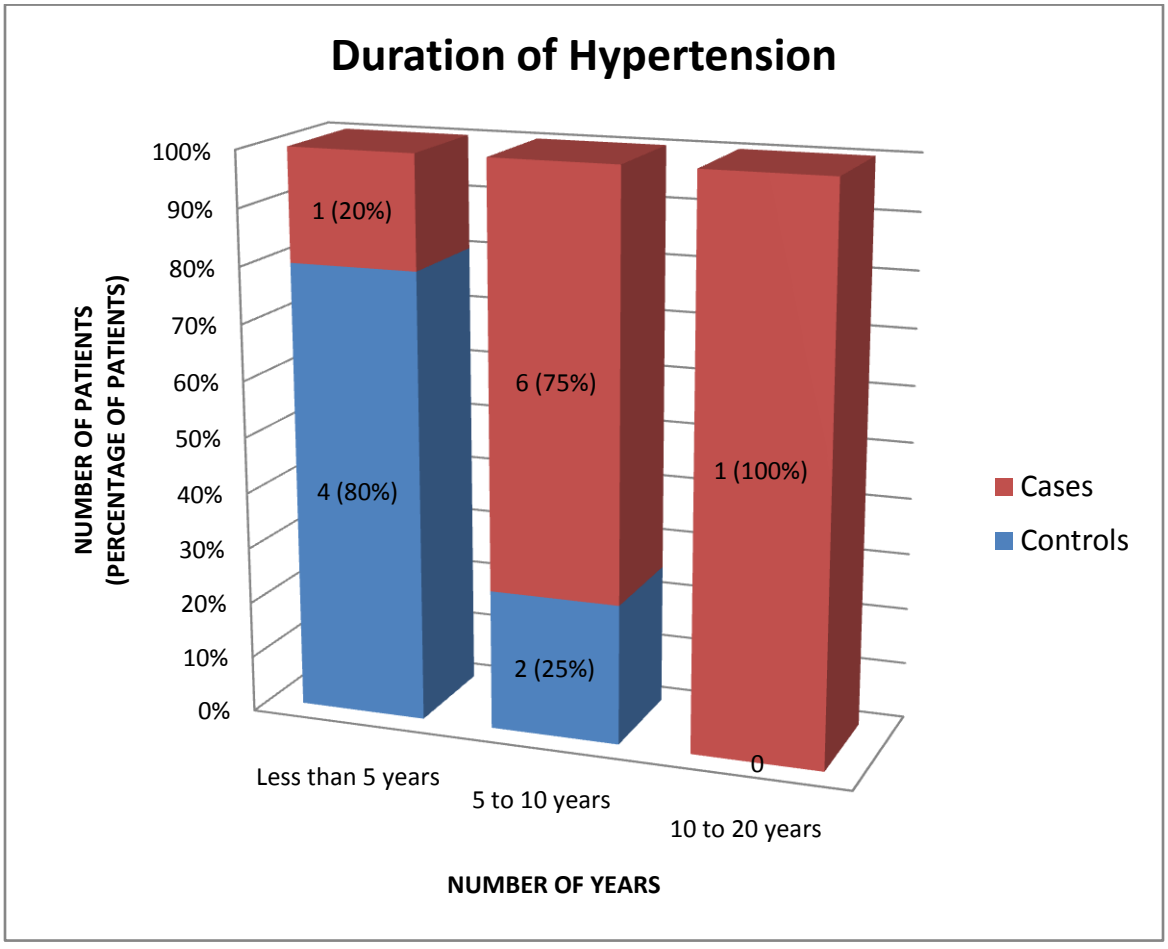


Figure 24:

The following graph indicates the presence of oral cancers as compared to the duration of hypertension among the cases and controls.



The following charts represent the characteristics of the oral carcinoma in the group of patients with oral cancer in our study population. This is a way to know the patient profile of the patients who come to us with oral cancers. Understanding the kind of patients that we see in our OPD can help us cater to their needs better.

Figure 25:

This following chart divides the oral cancer based on it's site in the oral cavity.

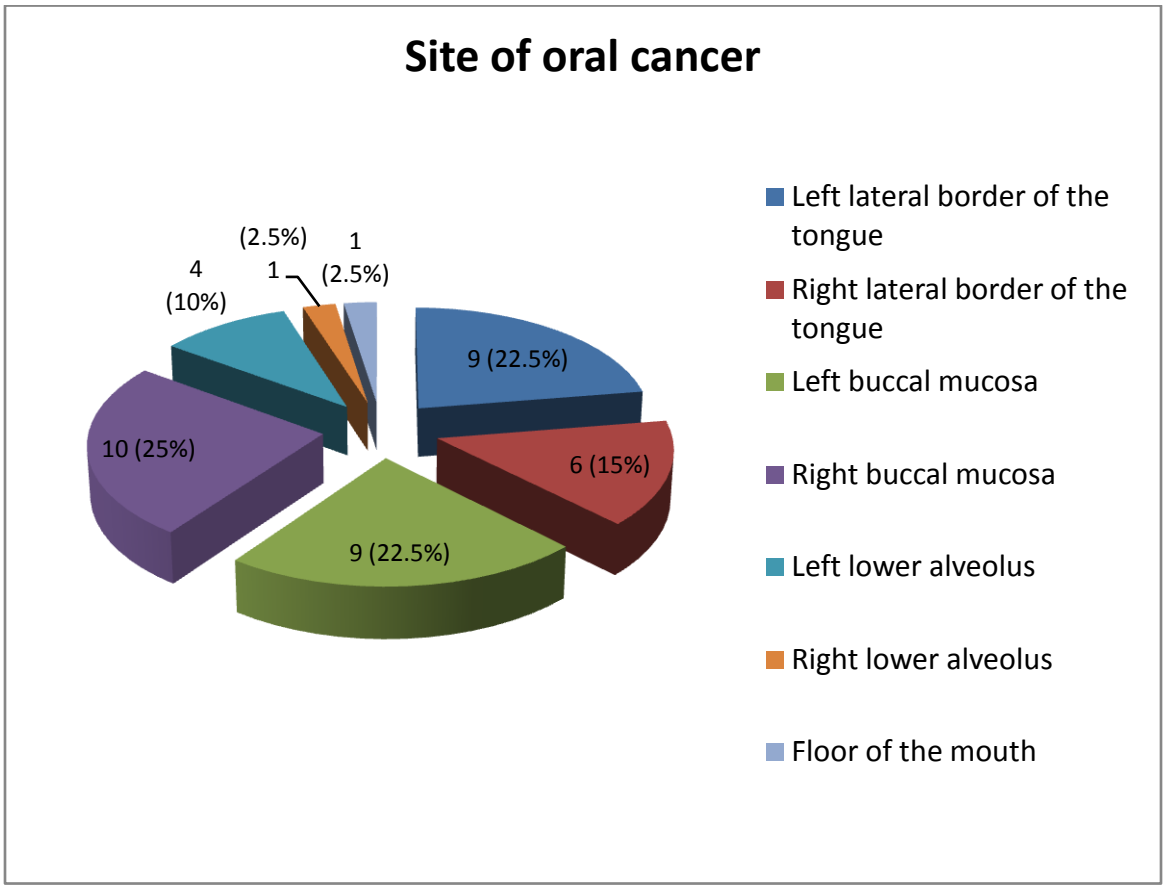


Figure 26:

This figure divides the oral cancers based on their size.

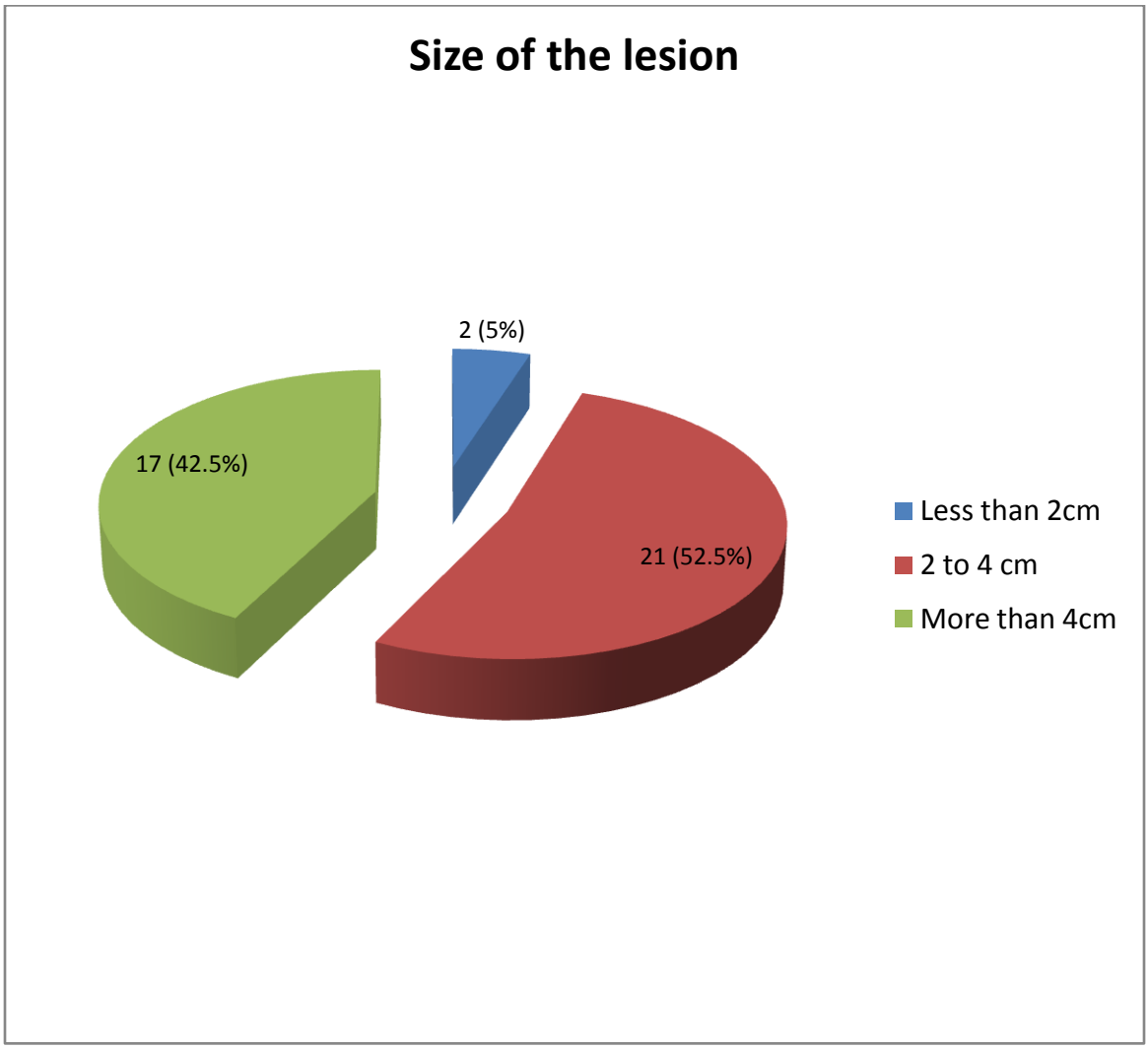


Figure 27:

Presence of lymph nodes.

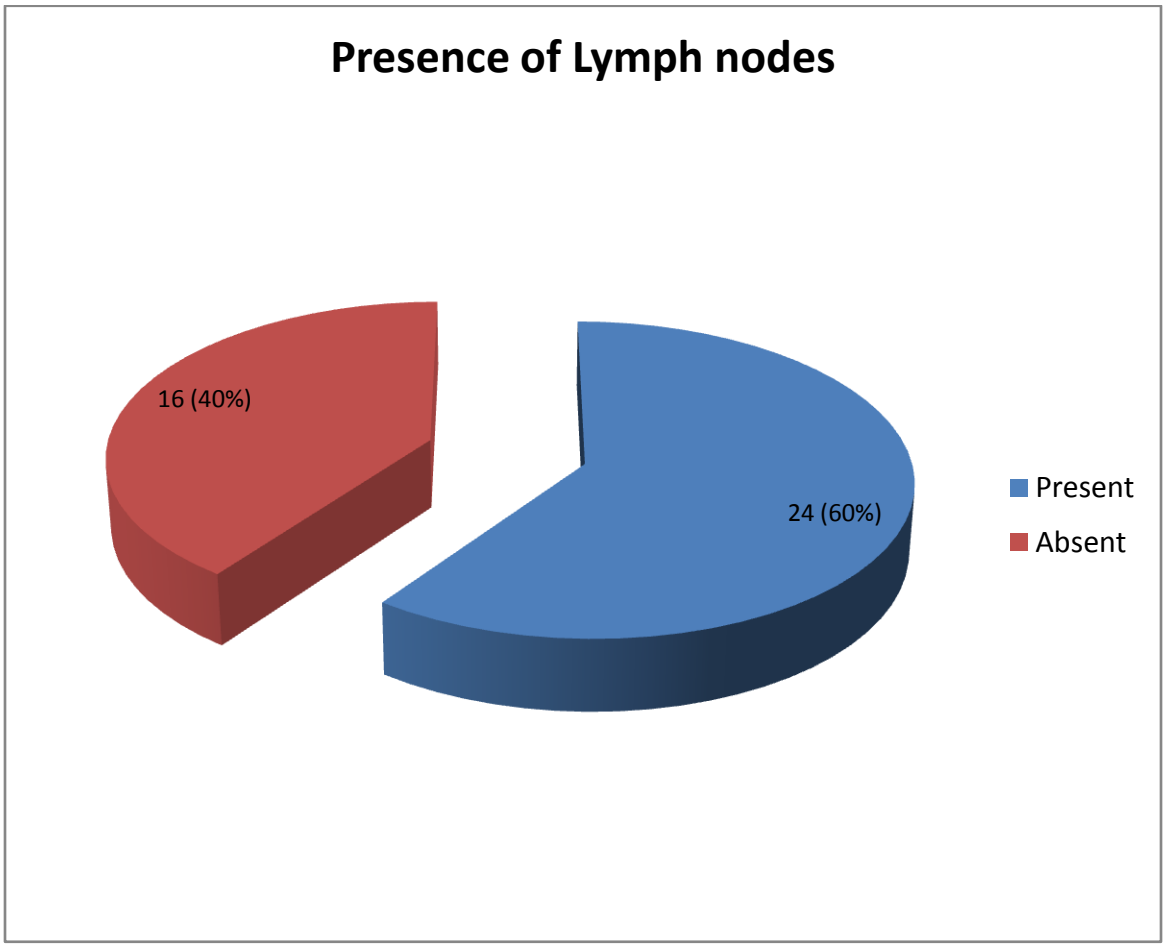


Figure 28:

The next slide shows the distribution of cases depending on the lymph nodal status.

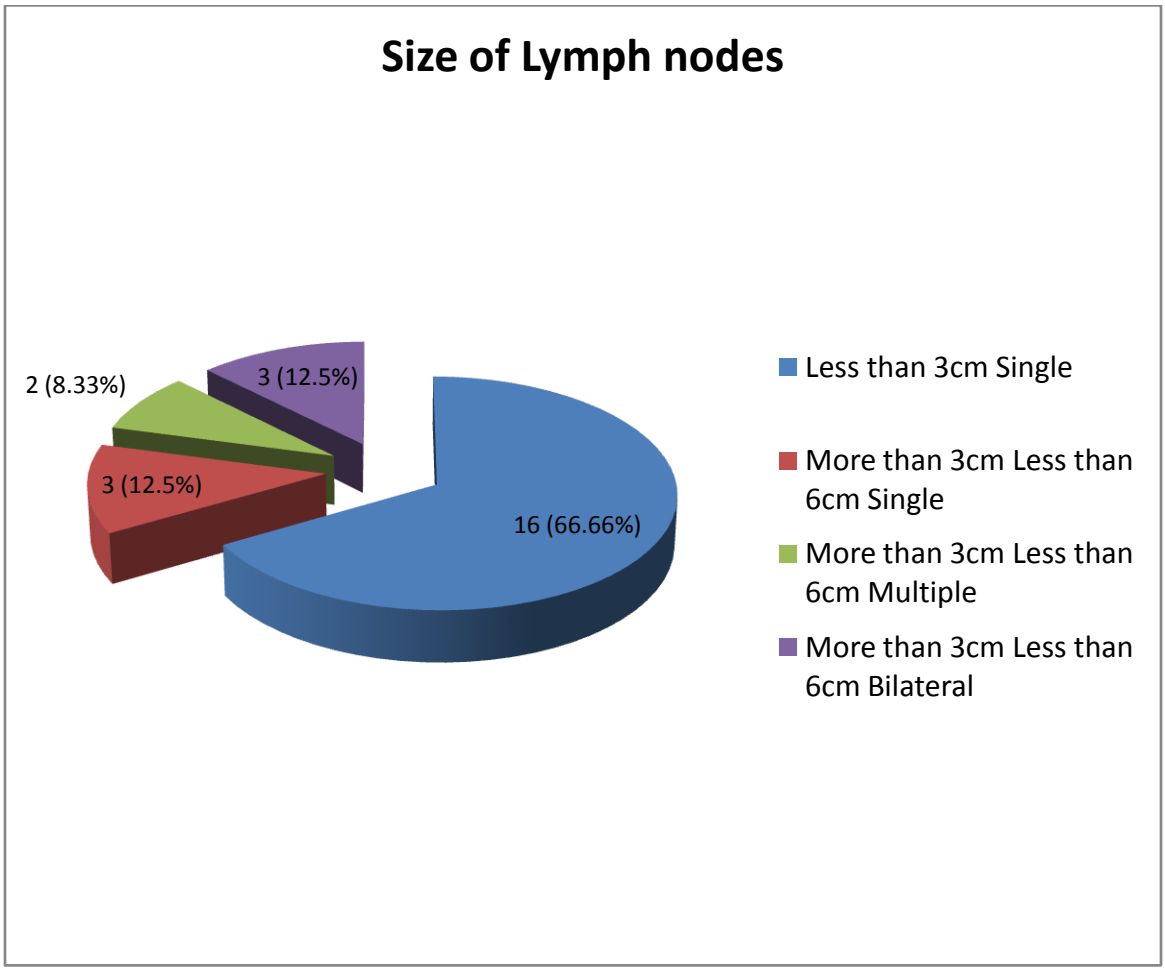


Figure 29:

The following table shows the distribution of oral cancers based on their degree of differentiation.

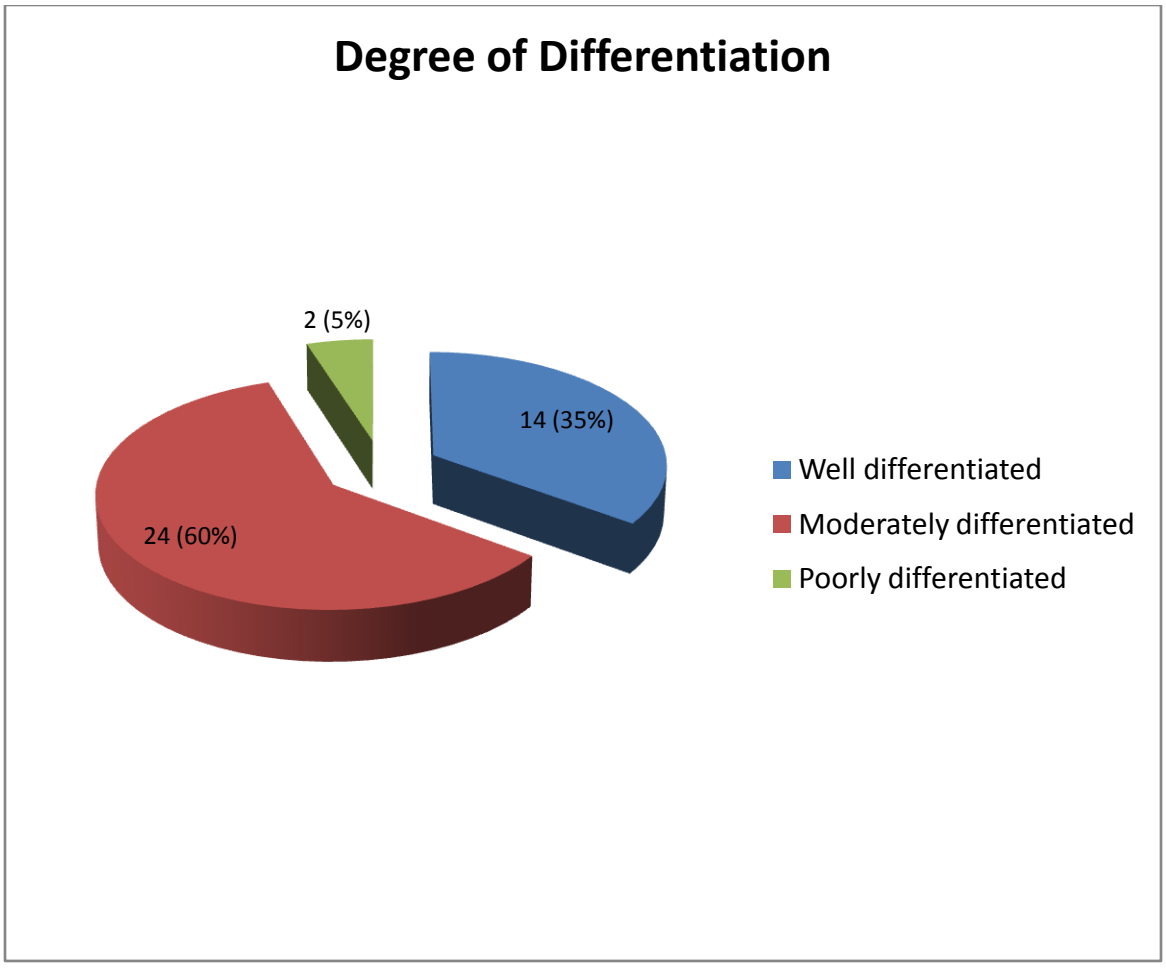
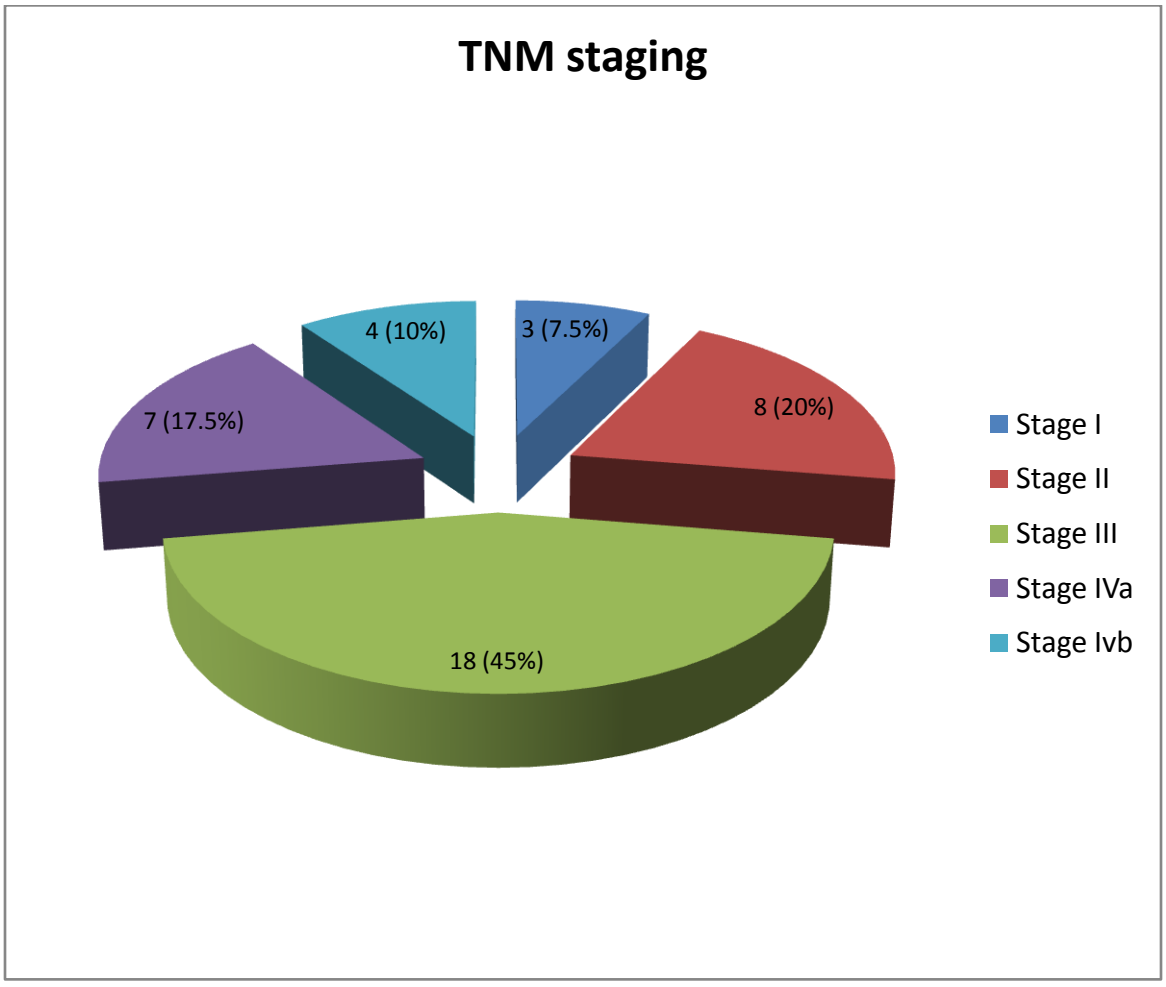


Figure 30 :

The following figure shows the distribution according to TNM staging.



ANALYSIS

The following was analysis based on the data collected. The gender distribution of the cases was studied. It was seen that 60% of the cases were men and only 40% were women. This corresponds to the observed pattern both in India and elsewhere in the world[37]. There has been a male preponderance in the incidence of oral cancers. The trend is changing slightly with an increase in smoking among women. But there is still a higher rate of oral cancers among men as compared to women. From the above analysis, it was seen that the patient profile of those with oral cancers who presented to the General Surgery OPD in our hospital was comparable to the data elsewhere. It was seen that there was a slight male preponderance in the number of patients with oral carcinoma which was comparable to the data worldwide[2],[3].In India, males are affected twice as much as females.[41]

The demographic pattern of the patients visiting the General Surgery OPD was also studied. It was seen that most of the patients were from South India, mostly from Tamil Nadu and Andhra Pradesh. There were also a large percentage of patients who had come from West Bengal. This distribution was similar to the patient profile generally seen in the General Surgery OPD in our hospital. The majority of patients who attend the outpatient department in our Institution are either from Tamil Nadu or West Bengal. Hence this may represent the patient profile of the outpatient department rather than the distribution of cases. The highest number of

the patients with oral cancers were from Tamil Nadu and the next highest were from West Bengal. This may be a reflection of the patient profile of our hospital rather than the distribution of oral cancer. The values were not significant in this case.

The educational status of the patients was also looked at. This was done to look for differences in the incidence of oral cancers based on the level of their education. Since education would be an indirect indicator towards the socioeconomic status, the level of education can be used to assess the socioeconomic status as well. But the level of education was the same in both the cases group and the control group. The values were not statistically significant ($p = 0.389$).This value may be a representation of the profile of patients that come to the General Surgery OPD of the Christian Medical College and Hospital. They may not represent those with oral cancers alone. It was seen that most patients had primary education. There was no significant difference in the education status of the cases and the controls.

The fact that this was comparable among the cases and controls may be indicative of the level of education in the country.

The next parameter that was looked at was the prevalence of smoking among those with oral cancers and those without oral cancers. This shows a slightly increased number of oral cancers among those who smoked. Though the percentage of smokers was slightly higher, this difference was not found to be statistically significant. ($p = 0.292$).

There was a definite increase in the incidence of oral cancers in those who smoked more than 10 cigarettes a day[38]. Though the values were not statistically significant ($p = 0.072$), seven out of the twelve who smoked more than 10 cigarettes per day had oral

cancer.(58.3%). Those who smoked both beedis and cigarettes had a slightly higher incidence of oral cancers which may be a cumulative effect of both beedis and cigarettes. One patient who smoked cigars was found to have oral cancer.

As could be expected, there was an increased incidence of oral cancer among those who smoked for longer periods of time. But, once again, the values were not statistically significant.($p = 0.733$)

The habit of consuming alcohol was found to be statistically significant in our study group ($p = 0$). This proves the fact that alcohol plays a causative role in oral cancers[39]. But there was no significant statistical difference that could be found among the cases and controls based on the frequency of consumption of alcohol ($p = 0.363$). There was no significant difference depending on the type of alcohol that was consumed either. Though it was not statistically significant it was seen that among those who consumed alcohol for less than 5 years only about 50% developed oral cancers. This percentage increased to 57% among those who consumed for 5 to 10 years. It was 100% among those who had been consuming alcohol for more than 10 years.

The significant difference was seen among the consumers of alcohol. There was an increased incidence among those who consumed alcohol for more than 20 years. But this value was not significant in the shorter duration. The same was true for chewing and smoking of tobacco where the values were more significant when the duration of the habit was more than 20 years.

The known causative factor that was studied was the chewing of tobacco[40]. There was some statistical significance for the chewing of tobacco and the causation of oral cancers. ($p = 0.006$). Those who chewed more often in one day (more than ten times a day) were found to be more affected (87.5%). There was no significant difference in the type of tobacco that was chewed but those taking both *Khaini* and *Ghutka*were found to be more commonly affected as compared to those who chewed either one or plain betel nut.

Another factor that was seen to make a difference was the number of years of chewing tobacco. About 81% of those with oral cancers were found to have been chewing tobacco for more than twenty years. Even though this was not statistically significant, ($p = 0.325$) the effect of tobacco, smoked and smokeless is well documented.

The number of patients who had risk factors of chewing or smoking tobacco were more in those who had oral cancers but these numbers were no significant. This may be because of the small sample size. This is comparable to the Indian statistics which show that Tobacco use and alcohol are known risk factors for cancers of the oral cavity. In India 57% of all men and 11% of women between 15-49 years of age use some form of tobacco.[42]

Oral hygiene of patients was also looked into. There were two patients who claimed they did not brush their teeth every day. Both were in the group of patients with oral cancers. The majority of patients brushed their teeth once a day. There was an equal distribution

of cases and controls among this group. There was no significant difference among those who brushed their teeth twice daily. Four (10%) had oral cancer among those who brushed twice daily while seven (17.5%) did not. This was not statistically significant(p= 0.063)

Most patients did not have a dental visit before the diagnosis was made. There were very few patients who had more than one dental visit before diagnosis. More than 50% of patients did not have dental visits prior to their diagnosis. This may be the reason for many tumours presenting at a more advanced stage. They may have had premalignant lesions which could have been picked up earlier, or treated early. Regular dental check up for all patients should be proposed. There was no significance among cases and controls in regard to their dental visits, statistically (p=0.756)

Most cases and controls said they had no dental problems. The commonest problem that was seen was caries teeth. Presence of dental problems was almost equal among cases and controls. Lack of dental problems or, rather lack of awareness of dental problems could also be a contributory factor to the delayed recognition of oral malignancies. If the patients had had regular dental visits or had consulted a dentist for their dental problems there could be early identification of lesions. Sharp teeth and oral cavities were seen among a few but the numbers were not significant. Only one patient among those with oral cancer had dentures. Since the number is small, significance could not be derived.

We also looked into other chronic illnesses the patient might have like, Diabetes Mellitus and Hypertension. Though the values were not found to be statistically significant ($p = 0.292$) longer duration of diabetes was found to correlate with presence of oral cancers. However, patients with 10 to 20 years of diabetes had oral cancer in 33.33% of cases while only in 12.5% of the controls. But, this value also proved to be statistically not significant.($p=0.267$)

The number of patients with hypertension in the cases and the control group was almost the same. There was no significant difference in both groups. Here again it was seen that those with longer duration of hypertension seemed to have increased incidence of oral cancers. Whether this was due to the co-existence of diabetes or due to the increased age of patients in this group was not clear.

Presence of Diabetes and hypertension was seen and analysed to look for a relationship between such chronic illnesses. Chronic diabetics were found to be more among the cases than the controls.

This may be due to the fact that diabetes seems to promote the RAS/RAF/MAPK signal transduction pathway mainly by the induction of erbB2 and erb B3 receptors leading to increased cell proliferation leading to carcinogenesis[43]. Diabetes also enhances the expression of H-ras and suppresses the expression of EGFR leading to increased cell proliferation. The pathways are presumed to lead to carcinogenesis[44]. But these values were not found to be statistically significant in our study.

The most common site of oral cancers seen in our centre was in the buccal mucosa followed by the lateral borders of the tongue.

The percentages were as follows :

Left lateral border of tongue : 22.5%

Right lateral border of tongue : 15%

Left buccalmucosa : 22.5%

Right buccalmucosa : 25%

Left lower alveolus : 10%

Right lower alveolus: 2.5%

Floor of the mouth 2.5%

The patients had presented to us mostly in Stage III with atleast a single node palpable. Most tumours were moderately differentiated. The majority of the lesions were between 2 to 4 cms.

It was seen that most tumours that present to us in the OPD belong to size between 2 to 4cm. This was followed by lesions that were more than 4 cm .The lesions that present in early stages where the tumour size was less than 2cm were relatively rare. Lesions less than 2 cm constituted 5% and those between 2 to 4 cm were 52.5%. Lesions more than 4 cm were 42.5%. This may be classical of the patients presenting to General Surgery OPD in our centre.

Nodes were found at presentation in the majority of the patients. 60% of the patients had atleast one node palpable at presentation. This again indicates the advanced stage at which our patients present to us .

From the graph it is seen that though most patients present with nodes, it was generally less than 3 cm. Only 3 patients of 24(12.5%) with nodal metastases had more than 3cm

nodes which were bilateral. Most patients presented with nodes less than 3 cm and single which was 66.66%. Nodes that were more than 3cm less, than 6 cm and single were 12.5%; more than 3 cm, less than 6 cm multiple were 8.33% and more than 3 cm less than 6 cm and bilateral were 12.5%

This hospital-based case-control study evaluated the association between a marker for HPV infection and oral cavity squamous cell cancer (SCC) at a tertiary hospital setting in southern India. Using the highly sensitive PCR, HPV DNA was examined in exfoliated cells of the buccal mucosa. We enrolled 40 case patients and 40 controls and used similar procedures for data and specimen collection, and for HPV testing. We found a nil prevalence of HPV DNA both among the cases and controls. The PCR testing was done in the oral mucosal scrapings of both the cases and controls. Further typing of HPV was to be done in samples that were positive for HPV. The cases and the control samples in this study selection did not have any positive samples for the presence of HPV.

Differences in the distribution of other known risk factors for oral cavity SCC, specifically tobacco smoking, alcoholic beverage consumption and betel chewing, between cases and controls were unremarkable.

The samples taken from both the cases and the controls were not positive for Human Papillomavirus. Patients in both the cases group and the control group were found to be negative for the virus in PCR studies. This was in contradiction to the results obtained from elsewhere in the world. The positivity for HPV in the normal mucosa is expected to be around 5% with incidence increasing in pre malignant lesion. The incidence of HPV in the oral mucosa of patients with oral squamous cell cancers was expected to be around 40% based on studies

done elsewhere in the world. The lack of positive result may indicate a different incidence of Human Papillomavirus in India.

The sample collection was done only by the principle investigator to make sure adequate sample was being collected by a uniform procedure in all the patients, both cases and controls. The samples were transported as soon as possible in ice packs to the virology department where they were refrigerated at 4^0c to make sure the sample was viable. Hence, there have been no issues with the method of sample collection or with the storage of the specimen.

The confirmation of the adequacy of the sample was done by the betaglobin levels in each of the samples[68]. The betaglobin level in each of the samples was adequate. Therefore, the sample collection was adequate.

The samples were analysed by PCR. The methodology has been explained in detail. This was the standard method and there were no problems in the execution of the tests. The PCRs were confirmed using housekeeping genes. There were both positive and negative controls. RT – PCR testing was done which is considered to be the most sensitive test for DNA viruses. The results of similar PCR testing done for Human Papillomavirus done in cervical mucosa has shown excellent detection rates in carcinoma cervix in our laboratory. Since the laboratory in our Institution has WHO authorization, there is no doubt about the validity of our testing process.

The kit that was used had a Collection Device which was HybriBio cell sampler (HybriBio Limited, Hong Kong).

The extraction kit used was HybriBio Extraction (HybriBio Limited, Hong Kong).

PCR amplification : a) Primers used : PGMY 09/11 (Target size – 450bp)[45]

b) Housekeeping genes :Betaglobin (Target size – 230bp)[46]

The sample that was chosen to be studied was oral mucosal scrapings. Oral mucosal scrapings have been proven to be representative of the flora in the oral cavity[68]. Hence, there has been adequate specimen collection and the method of collection and analysis has also been satisfactory.

This leaves us with the question of what the real reason for the lack of positive samples could be. This may indicate a different pattern or trend among our patient population. Most of the patients were from low to middle socio economic strata and belonged to various states from across the country. The differences in the climatic and geographic distribution of the patients may also play a part in the absence of HPV in this sample population.

There may different ethical, cultural and food habits in different areas. These may also be important in the causation of oral cancers. Some of these habits may also e protective in varying degrees.

We provide a brief review of statistics related to HPV and oral cavity SCC followed by a discussion of our study results.

During the last 15 years, HPV, the necessary cause of cancer cervix, has been causally linked with HNSCCs. The association is strongest for oropharyngeal cancer than oral cavity or laryngeal SCCs (IARC, 2007)[47]. Kreimer et al. (2005) in their review of HPV in HNSCCs worldwide (60 studies from 26 countries) found a significantly lower prevalence of HPV in oral cavity (23.50% of 2.642 cases; 95% CI=21.9-25.1) or laryngeal SCCs (24.0% of 1,435; 95% CI=21.8-26.3) as compared to oropharyngeal SCC (35.6% of 969; 95% CI=32.6-38.7)[48].

Molecular evidence provides support for certain types of HPV in the pathogenesis of a oral cavity SCC. The IARC (2007) in their evaluation of HPV and oral SCC concluded that there is "sufficient evidence in humans for the carcinogenicity of HPV 16 in the oral cavity," a conclusion based on strong evidence in exposed humans and sufficient evidence of carcinogenicity in experimental animals. They also concluded that there was "limited evidence in humans for the carcinogenicity of HPV 18 in the oral cavity," a conclusion based on limited evidence in humans and sufficient evidence of carcinogenicity in experimental animals (IARC, 2007)[47]. Kriemer et al. (2005) report that HPV16 accounted for a larger majority of HPV-positive oropharyngeal SCCs (86.7%; 95% CI= 82.6-90.1) compared with HPV-positive oral SCCs (68.2%; 95% CI=64.4-71.9) and laryngeal SCCs (69.2%; 95% CI=64.0-74.0). Conversely, HPV18 was rare in HPV-positive oropharyngeal SCCs (2.8%; 95% CI=1.3-5.3)

compared with oral cavity (34.1%, 95% CI=30.4-38.0) or laryngeal SCCs (17.0%, 95% CI=13.0-21.6). Aside from HPV16 and HPV18, other oncogenic HPVs were rarely detected in HNSCC. It was noted that tumor site-specific HPV prevalence was higher among studies from North America compared with Europe or Asia[48].

While the results of our study may reflect the true association of HPV and oral cavity SCC in our setting, it is plausible that the findings may in fact be due to an alternate explanation (Hennekens, 1987)[49]. Such alternate explanations may be due to the effects of bias, confounding or random error, which may produce spurious results leading to an absence of an association when it truly exists.

Bias

Perhaps the most serious problem hampering the validity of epidemiologic studies is the effect of measurement error in study variables. We do not perceive this to be a major source of bias in this study. In our study, the chance of bias was decreased by using an accurate method of identification of Human Papillomavirus. With regard to the detection of HPV, our use of PCR techniques and liquid-phase, immunocaptured hybridization has eliminated the severe misclassification of HPV status by the first generation of molecular epidemiology studies[50,51] (Franco, 1991; Schiffman and Schatzkin, 1994).Kreimer et al. (2005)[48] noted that HPV-16 and HPV-18 accounted for 16% and 8%, respectively of cases with HPV-positive oral cavity SCC; a slightly higher proportion of HPV prevalence may be detected by the inclusion of testing for other high-risk types of HPV found in the oral cavity such as types 31, 33, 35, 56, 58 and 68 which accounted for another 1.5%.

We did not perform serological tests to corroborate the negative HPV results in this study. A high prevalence of serum antibodies to HPV-16 oncoprotein E6 and E7, using enzyme-linked immunosorbent assay (ELISA), has been found to be a useful marker of past HPV-16 exposure (Viscidi et al., 2003)[54]. D'Souza et al. (2007) in a US-based case-control study of HPV and oropharyngeal cancer (cases, n=100; controls, n=200) found a prevalence of 72% of HPV-16 DNA in tumor specimens; serum antibodies against HPV-16 oncoprotein E6 or E7, or both were found in 64% of case patients and in 4% of control patients. With regard to negative HPV results, 28% of case patients had a negative HPV-16 DNA in tumour specimens, while 36% of case patients had a negative HPV-16 E6 or E7 serologic status. Among controls, 96% were sero-negative for HPV-16 oncoproteins[55].

Another reason for the high negative PCR results may be that the population studied may have lesser degree of high risk sexual behavior as compared to that of the various other populations studied in other studies. High-risk sexual behaviors have been found to be associated with HPV. Gillison et al. (2000) detected HPV more commonly among patients with more than one sexual partner and from those who practiced oral sex[56]. WHO data on sexual health behaviour indicators in India is scarce (WHO, 2010)[57]. However, the few available studies suggest that oral sex is not a popular practice due to the cultural bias against such behaviors (Avasthi et al., 2008)[58]. Other risk factors for increased risk of HPV infection such as premarital sex and having multiple partners are also less prevalent in India (15% -20%)[59,60] (Burchell et al., 2006; Joshi and Chauhan, 2011) as compared to the prevalence noted in western countries (75%)[61] (Finer, 2007). Because of the sensitive nature of questions and the potential ensuing psychological risk compounding the psychological trauma due to cancer diagnosis, we did not elicit detailed history on sexual behavior among the study group. But it has been clear from the

above mentioned studies that the population under study may differ from their counterparts in other studies in their sexual behavior.

Since ours was a case-control study, the chances of outcome miscalculation was very less. With regard to the outcome, case-control studies are unlikely to be affected by outcome misclassification as in cohort studies which are prone to this error as pre-invasive conditions may be classified as disease end points. Kriemer et al. (2005) suggest that misclassification of advanced oropharyngeal SCCs as oral cavity SCCs may have inflated the prevalence of HPV infection in oral cancers in Asia[48]. All cases in this study were biopsy confirmed, and did not include pre-invasive conditions such as leukoplakia. Most cases (45%) were in Stage III of the oral cavity SCC at the time of diagnosis, minimizing the bias due to misclassification.

Other studies have focused on the areas of the oral cavity that have higher predilection for HPV[55]. In the present study, the case group comprised of oral cavity cancer which included tumours of the lips, floor of the mouth, gum, palate, the anterior two-thirds of the tongue and the floor of the mouth below the tongue. Previous studies have shown that the most common sites for HPV-related head and neck cancers are cancers are the tonsil or base of the tongue (Syrjänen, 2005; Auluck et al., 2010) which are parts of the oropharynx. HPV infection was less strongly associated with other oral sites, such as the ventrolateral tongue, gingivae, cheek, palate, or floor of the mouth, where tobacco and alcohol are major etiological factors (Auluck et al., 2010)[62,63]. A case-control study of HPV and oropharynegal cancer byD'Souza et al. (2007) minimized selection bias by restricting case enrollment to primarily those with tumors located on the tonsil or base of tongue found a HPV prevalence of 40%[55]. Our studywas specifically designed to evaluate the association between HPV infection and oral cavity SCC.

Oral SCCs are more heterogenous with regard to the tumour location, anatomical sites, and are less likely to harbor the HPV than oropharyngeal SCC. Hence, in our study we have widened the area of the search for the Human Papillomavirus. The fact that we did not find positive results shows

Confounding

Tobacco smoking (IARC, 2004) or chewing (IARC, 2007) and alcoholic beverage drinking are strongly associated with oral cavity cancer, with attributable fractions of about 90% (IARC, 2007). [64,47].We did not find significant differences in the distribution of these established risk factors among cases and controls in our study. However, some tumours occur in subjects who are not exposed to known risk factors, and only a fraction of exposed subjects develop tumours. This suggests that other exposures may be independently involved or act as co-factor

Random error

Although we noted nil prevalence in this study, it is not an implausible finding. The International Agency for Research on Cancer (IARC) noted wide variations in HPV prevalence in oral cavity SCCs with studies reporting estimates ranging from 0% to 100% (IARC, 2007)[47]. However, in their assessment of pooled data from different countries, they included only studies that had more than 40 cases and had tested for the presence of HPV using PCR methods. The prevalence in these studies ranged from 4% to 80%. Kreimer et al. (2005) state that small sample size and publication bias complicate the assessment of the prevalence of HPV in HNSCCs beyond the oropharynx; they note that HPV prevalence was inversely proportional study sample size notably in oral cavity and larynx SCCs[48]. Moststudies clustered

between 10 and 100 cases; only five studies of oral cavity SCC included more than 100 cases (Herrero et al., 2003; Schwartz et al., 1998; Smith et al., 2004; Nagpaletal., 2002; Chang et al., 2003)[52,53,65,66]. The larger studies tended to show an overall prevalence that was substantially lower than the average. For example, in the large IARC multicentric study, the prevalence of HPV in oral cavity SCC was 3.9% based on 766 cases from 9 countries[52] (Herrero et al., 2003), whereas a study in India reported a prevalence of 73.6% based on 91 cases [67](Balaram et al., 1995). These findings suggest a selection bias wherein cases were preferentially included in the studies or that only studies reporting a high prevalence were published. Thus, conservative estimates of HPV prevalence estimates would be more realistic in power computations for studies of HPV and oral cavity SCC.

We estimated the study size based on 40% HPV prevalence among cases and 10% in controls and α error of 5. In light of the very wide variation in prevalence estimates and that HPV estimates in literature are mainly influenced by small studies, future estimates of HPV prevalence for power computation should be rather high. Using a conservative estimate of HPV prevalence estimates such as 5% prevalence in cases and 1% among controls, a sample size of 285 cases and 285 controls would be required to detect an effect. In future studies, using a higher sample size may bring out hitherto unknown factors to light. The results of our study may, in fact be the actual prevalence of HPV in the oral cavity of the Indian population. There may be other factors which may be the reason behind the absence of Human Papillomavirus in the oral cavity of Indians. Factors like the dietary practices of the population and other cultural differences may also contribute to the absence of HPV in these people.

CONCLUSIONS

1. There may be different factors responsible for oral cancers in the patients who present to the out-patient department in the Christian Medical college, Hospital, Vellore as can be seen from our study. There may be other reasons for the absence of Human Papillomavirus in this sub-group of patients. The results may be representative of the population that comes to this out patient department for treatment.

2. It was seen that there was a male preponderance in the patients with oral cancers which is comparable to studies done elsewhere. The rising use of tobacco products among both sexes may decrease this difference in the coming years.

3. Though not statistically significant the demographic pattern of patients with oral squamous cell cancers matched those done elsewhere.

4. More studies can be done in the future which may indicate the other reasons as why Human papilomavirus was not found in the patients presenting to our OPD which will lead to a different approach to the diagnosis and the management of oral squamous cell cancers.

LIMITATIONS

1. The number of cases and controls were calculated based on the existing literature. The numbers came to approximately 36 in each arm as the incidence in the general population

was about 40%. Hence the sample size was small, thereby causing difficulties in the analyses of facts.

2. Another feature that restricted the number of cases that were included in the study was the cost of the kit and the PCR testing. Hence, the numbers were kept to the minimum possible.

3. The RT PCR results were negative, hence further correlation between various risk factors could not be studied in detail.

BIBLIOGRAPHY

1. Barwad et al., “Human papilloma virus associated head and neck cancer.”A PCR based study. Diagnostic cytopathology; vol 40, issue 10, pages 893 – 897.

2. Global cancer statistics, 2002.Parkin DM, Bray F, Ferlay J, Pisani P CA Cancer J Clin. 2005;55(2):74.
3. Spitz MR. Epidemiology and risk factors for head and neck cancer. SeminOncol 1994; 21:281
4. Oral Cancer in India: Learning from Different Populations. Cancer prevention 2010; issue 14
5. Andre K, Schraub S, Mercier M, Bontemps P. Role of alcohol and tobacco in the aetiology of head and neck cancer: a case-control study in the Doubs region of France. Eur J Cancer B Oral Oncol 1995; 31B:301.
6. Blot WJ, McLaughlin JK, Winn DM, et al. Smoking and drinking in relation to oral and pharyngeal cancer. Cancer Res 1988; 48:3282
7. Lewin F, Norell SE, Johansson H, et al. Smoking tobacco, oral snuff, and alcohol in the etiology of squamous cell carcinoma of the head and neck: a population-based case-referent study in Sweden. Cancer 1998; 82:1367.
8. Kato I, Nomura AM. Alcohol in the aetiology of upper aerodigestive tract cancer. Eur J Cancer B Oral Oncol 1994; 30B:75
9. The connection between Human Papillomavirus and oropharyngeal squamous cell carcinoma in the United Staets; implication for Dentists. J. Am. Dental Ass.2011 Sept, 142(9); 1005 - 6
10. Iribarren C, Tekawa IS, Sidney S, Friedman GD. Effect of cigar smoking on the risk of cardiovascular disease, chronic obstructive pulmonary disease, and cancer in men. N Engl J Med 1999; 340:1773

11. Randi G, Scotti L, Bosetti C, et al. Pipe smoking and cancers of the upper digestive tract. Int J Cancer 2007; 121:2049.

12. Proia NK, Paszkiewicz GM, Nasca MA, et al. Smoking and smokeless tobacco-associated human buccal cell mutations and their association with oral cancer--a review. Cancer Epidemiol Biomarkers Prev 2006; 15:1061.

13. Sapkota A, Gajalakshmi V, Jetly DH, et al. Smokeless tobacco and increased risk of hypopharyngeal and laryngeal cancers: a multicentric case-control study from India. Int J Cancer 2007; 121:1793.

14. Rosenquist K, Wennerberg J, Schildt EB, et al. Use of Swedish moist snuff, smoking and alcohol consumption in the aetiology of oral and oropharyngeal squamous cell carcinoma. A population-based case-control study in southern Sweden.ActaOtolaryngol 2005; 125:991.

15. Znaor A, Brennan P, Gajalakshmi V, et al. Independent and combined effects of tobacco smoking, chewing and alcohol drinking on the risk of oral, pharyngeal and esophageal cancers in Indian men. Int J Cancer 2003; 105:681.

16. Douglas. E. Moore, Walter. J. Psoter, Deborah Cleveland, Donald Cohen; Cancer causes control 2007, November 18(9) : 919 – 929.

17. Blot WJ, McLaughlin JK, Winn DM, et al. Smoking and drinking in relation to oral and pharyngeal cancer. Cancer Res 1988; 48:3282.

18. De Stefani E, Boffetta P, Oreggia F, et al. Hard liquor drinking is associated with higher risk of cancer of the oral cavity and pharynx than wine drinking. A case-control study in Uruguay. Oral Oncol 1998; 34:99.

19. Malignant potential of oralsubmucous fibrosis due to intraoral trauma.

Indian J Med Sci. 2000 May;54(5):182-7.

20. Rosenquist, "Risk factors in oral and oropharyngeal squamous cell carcinoma." – a population based case – control study in Southern Sweden. Swedish Dental J Suppl 2005; (179), 1 – 66.

[21.] Cruz et al., "Age-dependence of human papillomavirus DNA presence in oral squamous cell carcinomas." European Journal of Cancer, Part B; Oral Oncology, Volume 32, Issue 1, January 1996, pg 55 – 62.

[22.] Miller and Johnstone, "Human papillomavirus as a risk factor for oral squamous cell carcinoma." – a meta-analysis. 1982 -1997. Oral Surg Oral Med Oral Pathol Oral RadiolEndod 2001, 92: 170 – 179.

[23.] Miller and White, "Human papillomavirus expression in oral mucosa, premalignant conditions, and squamous cell carcinoma."Oral Surg Oral Med Oral Pathol Oral RadiolEndod 1998, July; 82(1), 57 -68.

24. Paz et al., “Human papillomavirus (HPV) in head and neck cancer. An association of HPV 16 with squamous cell carcinoma of Waldeyer’s tonsillar ring.”

25. Sturgis EM, Ang KK. The epidemic of HPV-associated oropharyngeal cancer is here: is it time to change our treatment paradigms? J NatlComprCancNetw 2011; 9:665.

[26.] Chaturvedi A, Engels EA, Pfeiffer RM, et al..Human papillomavirus and rising oropharyngeal cancer incidence in the United States.J ClinOncol 2011.

[27.] Tilston P. Anal human papillomavirus and anal cancer. J ClinPathol 1997; 50:625.

28. Electron micrograph of a negatively stained Human Papillomavirus; Rating HPV biomarker in Head and Neck cancers; Sept 18, 2012, news; brown.edu.

29.D’Souza et al., “Case–Control Study of Human Papillomavirus and Oropharyngeal Cancer.” N Engl J Med 2007; 356 : 1944-56

30. Schiffman M, Wentzensen N, Wacholder S, et al. Human papillomavirus testing in the prevention of cervical cancer. J Natl Cancer Inst 2011; 103:368

31. Human papillomavirus – associated head and neck cancer is a distinct epidemiologic, clinical, and molecular entity. Seminars in oncology. Volume 31, Issue 6 , December 2004, Pages 744 -754.

32. Chaturvedi A, Engels EA, Pfeiffer RM, et al..Human papillomavirus and rising oropharyngeal cancer incidence in the United States.J ClinOncol 2011.

33. Andisheh-Tadbir, Mehrabani, and Heydari, “Sociodemographic and etiological differences of head and neck squamous cell carcinoma in young and old patients in southern Iran.”Journal of craniofacial Surgery; January 2010 – volume 21 – issue 1; pp 126 – 128.

34. Human Papillomavirus in cervical and head and and neck cancers. Amanda Psyrri and Daniel Dimaio. Nature Clinical Practise, Oncology(2008) 5, 24 -31.

35. Human papillomavirus testing and molecular markers of cervical dysplasia and carcinoma; Donna Dehn, Kathleen C. Torkko, Kenneth R. Shroyer, Cancer cytopathology, Volume 111, Issue 1, Pages 1 – 14. February 2007.

36. C. A. Heid, J Stevens, K. J. Livak and P.M. Williams; Genome research; 1996: 6: 986 – 994.

37. Gillison ML, D'Souza G, Westra W, et al. Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. J Natl Cancer Inst 2008; 100:407.

38. Kreimer et al., “Human papillomavirus types in head and neck squamous cell carcinomas worldwide.” : A systematic review, Cancer Epidemiology, Biomarkers and prevention; February, 2004, 14:467.

39. Feller et al., “Human papillomavirus-mediated carcinogenesis and HPV-associated oral and oropharyngeal squamous cell carcinoma. Part 2.” Head and Face Medicine 2010, 6:15

[40.] Zheng et al., "The screening of viral risk factors in tongue and pharyngolaryngeal squamous carcinoma." International Journal of Cancer Research and Treatment, April 2010, Volume 30, no.4, 1233-1238 .

41. Infectious and dietary risk factors of oral cancer.Oral oncology 2010 Jun;46(6):411-3.

42. Fakhry et al., "Associations between oral HPV16 infection and cytopathology."Evaluation of an orophayngeal "pap test" equivalent in high risk populations.Cancer Prevention Research; Sept 2011, 4; 1378.

43. Diabetes and Oral Oncogenesis; Anti cancer J 2007; Nov – Dec 27(6b) 4185 – 93.

44. Diabetes and cell proliferation, Histopathology 2009 May 24(5) 531 -9.

45. Haws et al; Journal of Virological Methods, 122, 2004; 87 – 93.

46. Gravitt et al, JCM, 2000, 38(1); 357 – 61.

47. IARC. 2007. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 89, Smokeless tobacco products, Lyon

48. Kreimer AR, Clifford GM, Boyle P, Franceschi S. 2005. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev 14: 467-475.

49. Hennekens CH, Buring JE. Epidemiology in Medicine, Lippincott Williams & Wilkins, 1987.

50. *Franco EL.* 1991. *The sexually transmitted disease model for cervical cancer: incoherent epidemiologic findings and the role of misclassification of human papillomavirus infection.* Epidemiology2*:*98-*106.*

51. *Schiffman MH, Schatzkin A.* 1994.*Test reliability is critically important to molecular epidemiology: an example from studies of human papillomavirus infection and cervical neoplasia.* Cancer Res54(7 Suppl)*:*1944s-*1947s.*

52. Herrero R, Castellsague X, Pawlita M, Lissowska J, Kee F, Balaram P, Rajkumar T, Sridhar H, Rose B, Pintos J, Fernandez L, Idris A, Sanchez MJ, Nieto A, Talamini R, Tavani A, Bosch FX, Reidel U, Snijders PJ, Meijer CJ, Viscidi R, Munoz N, Franceschi S, Group IMOCS. 2003. Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. J Natl Cancer Inst 95: 1772-1783.

53. Schwartz SM, Daling JR, Doody DR, Wipf GC, Carter JJ, Madeleine MM, Mao EJ, Fitzgibbons ED, Huang S, Beckmann AM, McDougall JK, Galloway DA. 1998. Oral cancer risk

in relation to sexual history and evidence of human papillomavirus infection. J Natl Cancer Inst 90: 1626-1636.

54. Viscidi RP, Ahdieh-Grant L, Clayman B. Serum immunoglobin G response to human papillomavirus type 16 virus-like particles in human immunodeficiency virus (HIV)-positive and risk-matched HIV-negative women. 2003. J Infect Dis 187: 194-205.

55. D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM, Westra WH, Gillison ML. 2007. Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med 356: 1944-1956.

56. Gillison ML, Koch WM, Capone RB, Spafford M, Westra WH, Wu L. Evidence for a causal association between human papilloma virus and a subset of head and neck cancers. J Natl Cancer Inst 2000;92:709-20.

57. WHO/ICO HPV Information Centre. Human papillomavirus and related cancers, INDIA. Summary Report Update. September 15, 2010.

58. Avasthi A, Kaur R, Prakash O, Banerjee A, Kumar L, Kulhara P. 2008. Sexual behavior of married young women: a preliminary study from north India. Indian J Community Med 33(3):163-7.

59. Burchell AN, Winer RL, de Sanjosé S, Franco EL. Chapter 6: Epidemiology and transmission dynamics of genital HPV infection. Vaccine. 2006 Aug 31;24Suppl 3:S3/52-61.

60. Joshi B, Chauhan S. 2011. Determinants of youth sexual behaviour: program implications for India. Eastern Journal of Medicine 16:113-121

61. Finer LB. Trends in premarital sex in the United States, 1954-2003. 2007. Public Health Rep 122(1):73-8.

62. Syrjänen S. 2005. Human papillomavirus (HPV) in head and neck cancer. J ClinVirol 32 Suppl 1:S59-66.

63. Auluck A, Hislop G, Bajdik C, Poh C, Zhang L, Rosin M. 2010. Trends in oropharyngeal and oral cavity cancer incidence of human papillomavirus (HPV)-related and HPV-unrelated sites in a multicultural population: the British Columbia experience. Cancer 116(11):2635-44.

64. IARC. 2004. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 83, Tobacco smoke and involuntary smoking, Lyon.

65. Smith EM, Ritchie JM, Summersgill KF, Klussmann JP, Lee JH, Wang D, Haugen TH, Turek LP. 2004. Age, sexual behavior and human papillomavirus infection in oral cavity and oropharyngeal cancers. Int J Cancer 108: 766-772.

66. Chang JY, Lin MC, Chiang CP. 2003. High-risk human papillomaviruses may have an important role in non-oral habits-associated oral squamous cell carcinomas in Taiwan. Am J ClinPathol 120: 909-916.

67. Balaram P, Nalinakumari KR, Abraham E, Balan A, Hareendran NK, Bernard HU, Chan SY. 1995. Human papillomaviruses in 91 oral cancers from Indian betel quid chewers--high prevalence and multiplicity of infections. Int J Cancer 61: 450-454.

68. BengtGöran Hansson PhD, Kerstin Rosenquist, Annika Antonsson, Bengt
Göran Hansson PhD, Kerstin Rosenquist, Annika Antonsson, Johan Wennerberg, Elsy-Britt Schildt, Anna Bladström&GunillaAndersson (2005) Strong association between infection with human papillomavirus and oral and oropharyngeal squamous cell carcinoma: A populationbased case-control study in southern Sweden, ActaOto-Laryngologica, 125:12, 1337-1344

APPENDIX

A: INFORMED CONSENT

A study is being conducted in Christian Medical College and Hospital in the General Surgery OPD to study the presence of Human Papillomavirus (HPV) in the oral cavity and to see whether it is related to the causation of cancer of the oral cavity.

The common causative factors of oral cancer are tobacco chewing and alcohol consumption. It has been seen in earlier studies that HPV also plays an important role in the causation of cancer. This study aims at evaluating the presence of HPV in the mouth and its association with oral cancers.

In this study, scrapings from your mouth will be taken using a small brush and the sample sent to the Virology Department. The presence of HPV will be evaluated using a kit and its type identified. You will also be required to fill a questionnaire in regard to your habits. Confidentiality will be maintained.

It has been seen that HPV can be found in the mouth of persons without any oral lesions. In that case, if you prefer it, the results of the study will be informed to you. You will have to have regular check up and evaluate immediately if you develop oral lesions.

The decision to participate in this study is purely voluntary. Your treatment will continue regardless of your participation in the study. Your decision will not affect your treatment in this hospital in any way.

If you have any doubts or questions, kindly contact, Department of General Surgery.

Christian Medical college and Hospital.Ph. No: 9944807738

B :DATA ENTRY FORM

EVALUATION OF RELATIVE RISK OF ORAL SQUAMOUS CELL CARCINOMA IN PATIENTS WITH HUMAN PAPILLOMA VIRUS

1. S. no.

2. Name : 3. Hospital number:

4. Age: 5. Sex:

6. Address:

7. Phone number:

8. Education:

9. Occupation:

10. State :

C: QUESTIONNAIRE FOR RISK FACTOR ANALYSIS IN ORAL CANCERS

1. Age

2. Sex 1.Male 2. Female

3.State 1. Tamil Nadu 2.Andhra Pradesh 3.West Bengal 4. North Eastern states

5. Others

4. Smoking History

a. Smoker 1. Yes 2.No

b. Smokes 1. Beedis 2. Cigarettes 3.Cigar

4.pipe 5. Reverse smoking

c. No. of years:

5. Alcohol consumption:

a.Consumer: 1Yes 2.No

b. Consumes: 1. wine 2. arrack 3.beer

c. Frequency :

d. Number of years:

6. Chews paan : a.1.Yes 2.No

b. Type of paan :

c. Frequency :

d. Number of years:

7. Dental hygiene :

a. Brushes teeth 1. Yes, twice daily 2. Yes, once daily 3. No

b. dental visits: 1.Yes, regularly 2.No.

c. History of caries teeth/ cavities.

d. Dentures: 1. Yes 2. No

8. Other co-morbid illnesses:

a. Diabetes : 1.Yes 2. No

b. Duration :

c. Hypertension: 1. Yes 2. No

d. Duration :

9. Site of the tumour.

a. buccal mucosa b. lateral border of the tongue

c. alveolus d. floor of the mouth.

10. Size of the tumour.

11. Degree of differentiation:

12. Presence of neck nodes:

13. TNM Staging